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TRANSPORT OF α-AMINOISOBUTYRIC ACID IN TOMATO PERICARP SLICES AND MICROSOMAL VESICLES.
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α-Aminoisobutyric acid (α-AIB) was used as a model substrate to study the transport of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), in tomato pericarp slices and microsomal vesicles. In pericarp slices, α-AIB (ACC) uptake is metabolically-dependent and passive with α-AIB (ACC) accumulating in the vacuolar compartment. In microsomal vesicles, α-AIB (ACC) uptake is passive with α-AIB (ACC) accumulating linearly with time of incubation. At present, a metabolically-dependent uptake is not observed even though the vesicles have an anion-sensitive, Mg-dependent ATPase activity which drives the formation of a pH gradient (acid inside) and a membrane potential (positive inside).

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ACTIVE CO_2 AND HCO_3^- TRANSPORT IN CYANOBACTERIA
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Cyanobacteria can actively transport both CO_2 and HCO_3^-. Transport of CO_2 is constitutive whereas transport of HCO_3^- is induced by growth at limiting concentrations of inorganic carbon. Transport of HCO_3^- by air-grown cells is inhibited by the absence of Na^+ or by Li^+ in the presence of Na^+. Transport of CO_2 requires much lower Na^+ concentrations. Transport of HCO_3^- is inhibited by monensin and amiloride, whereas CO_2 transport is not. Transport of CO_2 is selectively inhibited by carbon oxysulfide (COS) which is a structural analog of CO_2. The COS is a reversible and competitive inhibitor that is itself transported into the cells. The COS is converted by the cells to H_2S and CO_2, either during the transport process itself or by intracellular carbonic anhydrase. Transport of CO_2, but not HCO_3^-, is also inhibited by H_2S in a reversible fashion. The rate of CO_2 transport does not appear to be hindered when rapid HCO_3^- transport is initiated by the addition of Na^+. The results obtained with various inhibitors suggest that the CO_2 and HCO_3^- transport processes may not be functionally linked.

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THE 5'FLANKING REGION OF *rbc* IS INVOLVED IN THE ADAPTATION OF CYANOBACTERIA TO LOW-CO_2.

Devorah Friedberg, Rina Ariel, Leonora Reinhold, July Seijffers, Martin Kessel, Aaron Kaplan Life Sciences Institute, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.

The high CO_2 requiring mutants of *Synechococcus* PCC7942, E_1 and O_{221}, are capable of accumulating inorganic carbon internally like the wild type. These mutants possess aberrant carboxysomes. The mutations were mapped in the 5' flanking region of *rbc*. Insertional inactivation of the BstXI site, 1.4kb from the 5' of *rbcL* by a gene conferring kanamycin resistance, also produced high CO_2 requiring mutant. The significance of this region and of the carboxysomes and carbonic anhydrase for cyanobacterial photosynthesis will be discussed.

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REGULATION OF GENE EXPRESSION DURING PLANT DEVELOPMENT; GENERAL PROCESSES
R. B. Goldberg, Biology Department, University of California, Los Angeles, CA 90024
Abstract not available at press time.

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SITE-SPECIFIC MUTATIONS ALTER FACTOR BINDING *IN VITRO* AND CHANGE ORGAN SPECIFIC EXPRESSION IN TRANSGENIC PLANTS
Nam-Hai Chua, Eric Lam, Philip N. Benfey, Philip M. Gilmartin, & Rong-Xiang Fang Lab. Plant Molecular Biol., The Rockefeller Univ., 1230 York Avenue, NY, NY 10021-6399;
We have detected a protein factor (ASF-1) which binds to a tandemly repeated TGACG motif upstream of the cauliflower mosaic virus 35S promoter. Site-specific mutations in the binding site block binding of this factor *in vitro*. In protoplasts these mutations diminish expression of the 35S promoter. In transgenic plants expression of the 35S promoter is greatly reduced in roots. Conversely, the insertion of the 21 bp ASF-1 binding site into a green tissue-specific promoter element results in high expression in root. Thus, a factor binding site which is defined by site-specific mutations is shown to be sufficient to alter the organ-specificity of promoters *in vivo*.

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TRANSCRIPTIONAL REGULATION OF GENES DURING THE TRANSITION FROM EMBRYOGENY TO GERMINATION
J.J. Harada, L. Comai, R.A. Dietrich, M. Gomez-Pedrozo, and C.S. Baden, Department of Botany, University of California, Davis, CA 95616
To understand the cellular processes regulating the transition between embryogeny and postgerminative growth, we are studying the regulation of genes expressed in late maturation-stage embryos and in seedlings. We used cloned probes representing genes that are primarily expressed in either embryos (embryo-specific genes) or seedlings (postgermination-abundant genes) with transcription assays in isolated nuclei and showed that the majority of the genes are regulated at the transcriptional level. Differences in the transcriptional activity of embryo-specific and postgermination-abundant genes in nuclei from embryos, dry seeds, and leaves appear to define the timing of the shift between embryonic and postgerminative growth.
(Supported by NSF Grant No. DCB-8518182).

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CONTROL OF CHLOROPLAST GENE EXPRESSION

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Plastid gene expression in higher plants is regulated by a hierarchy of transcriptional and post-transcriptional control steps during plant development. Changes in the transcriptional activity during chloroplast development and plastid differentiation affects most genes similarly, and transcriptional regulation may be operational for only a few genes. Post-transcriptional regulatory events include processing of polycistronic transcripts, splicing of intron-containing mRNAs, and the differential stability of individual mRNAs. Superimposed on these controls, the light-dependent synthesis of photosynthetic proteins can also be regulated at the level of translation initiation and elongation. Our current understanding of the mechanisms involved in the various control steps will be reviewed and discussed in the context of plastid functions.

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PECTIC OLIGOMERS INDUCE INCREASES IN ETHYLENE, LYCOPENE, AND PHENOLICS IN TOMATO PERICARP DISKS

Alan D. Campbell and John M. Labavitch, Dept of Pomology, University of California at Davis, Davis, CA 95616

Pectic oligomers released from cell walls may play a role in the regulation of ripening and of response to wounding in tomato fruit. Pericarp tissue disks isolated from mature tomato fruit have been used to study the short- and long-term responses of tomato tissues to pectic oligomers, galacturonic acid, and neutral sugars, and to engogenous compounds released during ripening. Pectic oligomers, galacturonic acid, and endogenous compounds can induce both a short-term increase in ethylene biosynthesis and a long-term rise in biosynthesis of ethylene, lycopene, and phenolic materials. Neutral sugars produce no comparable effect. The transient increase in ethylene biosynthesis in tomato disks reaches a maximum about 4 hours after addition. The increases in biosynthesis of lycopene and of phenolic compounds are frequ ently detectable within 24 hours, but are dependent on the stage of maturity at treatment and on the conditions under which the disks are maintained. ACC treatments which produce a comparable rise in ethylene do not induce a comparable lycopene or phenolic response. The significance and interdependence of these, and other, responses to pectic oligomers during ripening are being explored.

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ALTERED DEVELOPMENTAL REGULATION IN CALYX OF *IN VITRO* TOMATO FRUIT

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In vitro tomato fruit were cultured from pollinated and unpollinated flowers of greenhouse-grown plants. Flowers were disinfested and the corolla, stamens, and most of the pedicel removed. Floral explants were grown on Murashige and Skoog salts, 6% sucrose, White's vitamins and glycine, and 100 mg/l inositol, pH 5.7, kept at 22° C, and illuminated for16 h/da. After approximately 6 wks, fruit ripened, and calyces became abnormally swollen and red. Calyces of undeveloped fruit were also abnormal. RNA was extracted from both red and green fruit and calyces. A cDNA probe for polygalacturonase (PG), an enzyme that in tomato is expressed only in ripening fruit (DellaPenna D *et al.* 1986 Proc Natl Acad Sci USA 83:6420-6424), hybridized to RNA from both ripe tomato fruit and red calyx tissue, but not to RNA from green fruit and calyx. Ultrastructural studies show membrane changes in both fruit and calyx tissue Calyx detached from the main floral explant also ripens. Thus, the developmental program of calyx tissue has been altered at least in some aspects to that of fruit, so that the calyx now ripens.This system should be useful to study possible trigger mechanisms of fruit ripening.

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JACK PINE FLOWERING - NITROGEN AND GIBBERELLIN INTERACTIONS

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Interactions of $GA_{4/7}$ biweekly sprays (400 mg/L) and different levels of soil-applied NH_4NO_3 were examined in jack pine seedling and clonal orchards and in potted trees under polythene shelters. Untreated trees in the seedling or clonal orchard produced similar numbers of female flowers, whereas male flower production was 5- to 9-fold higher in the seedling orchard. $GA_{4/7}$ and NH_4NO_3 treatments enhanced female flowering; the best combination was $GA_{4/7}$ with 400 kg N/Ha. It provided a 2-fold increase in the clonal orchard and a 4-fold increase in the seedling orchard. $GA_{4/7}$ alone increased male flower counts 3-fold in the clonal and seedling orchard. Without $GA_{4/7}$, optimum NH_4NO_3 for male flowering in both orchards was 200 kg N/Ha; with $GA_{4/7}$, male flowering was greatest with no added nitrogen. $GA_{4/7}$ enhanced male and female flowering in potted trees. Luxury levels of nitrogen slightly increased female flowering in the presence of $GA_{4/7}$, whereas nitrogen deficiency caused a massive increase in male flowering.

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MEASUREMENT OF ENDOGENOUS ABA IN BUDS OF *Tsuga heterophylla* TREATED TO MODIFY SEX DIFFERENTIATION

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Sex expression in conifers can be modified by cultural and hormonal treatments, but the basis is not clear. To modify male and female conebud production, pruning of the shoot, combined with "early-" or "late heat", with or without application of gibberellin $A_{4/7}$, was administered before budbreak and the initiation of reproductive apices in *T.heterophylla*. The endogenous ABA in the terminal and lateral buds harvested in late June was quantified by GC-MS-SIM. The ABA conc. was low (200 pg/mg d.w.) in potential conebuds from female pruning treatments, regardless of crown position or time of heating, but was about 70-fold higher in those from male pruning treatments. Applied $GA_{4/7}$ did not change the ABA level in male pruning treatments, but increased ABA conc. in female pruning treatments up to 100-fold. The implications of these results, and the results of IAA and GA analyses, will be discussed.

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GENETIC CONTROL OF FLORAL ORGANOGENESIS IN ARABIDOPSIS THALIANA

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We are studying the genetic control of flower development through analysis of Arabidopsis thaliana mutants. A number of mutants have been isolated in which one organ type is replaced by another organ type (homeotic mutants). One such mutant, Flo10, shows homeotic transformation of carpels to stamens, as a result of a single, recessive, nuclear mutation. Studies of the morphology, ontogeny and genetics of Flo10 and the possible function of the FLO10 gene product in determining reproductive organ identity will be presented.

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FLORAL DEVELOPMENT IN ARABIDOPSIS THALIANA: THE ROLE OF AP2 LOCUS

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The control of pattern formation during flower development is not well understood. In an attempt to investigate the genetic mechanisms involved in floral organ initiation and differentiation, we are studying mutants of Arabidopsis thaliana that exhibit homeotic changes in flower morphology. Three independent mutants (flo2, flo3, flo4) were isolated which cause homeotic transformations of the perianth: sepals to carpels and petals to stamens, but do not affect the morphology of the inner whorls (stamens and carpels). Genetic analysis has shown that all three phenotypes are due to recessive mutations at a single nuclear locus, AP2. The floral morphology and ontogeny associated with different alleles of the AP2 locus will be presented and the potential role of the AP2 gene product in floral development will be discussed.

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POLYAMINE METABOLISM DURING GROWTH AND DEVELOPMENT OF *IN VITRO* CULTURES OF RICE

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Polyamines (PAs) and enzymes of the PA biosynthesis pathway have been implicated in various morphogenic and developmental processes in a wide variety of species. In carrot cell cultures PA biosynthesis is required for somatic embryogenesis. We have investigated the role of PAs in callus growth, somatic embryogenesis, and plant regeneration from *in vitro* cultures of indica rice (*Oryza sativa* L.). Although addition of 10 to 1000 µM putrescine (Put), spermidine (Spd), or spermine (Spm) to the culture medium did not affect primary callus growth or plant regeneration significantly, callus growth was inhibited by 1.0 to 5.0 mM of diflouromethyl-arginine (DFMA), a specific inhibitor of arginine decarboxylase. Plant regeneration after three weeks of callus induction in medium containing DFMA or diflouromethylornithine (DFMO), a specific inhibitor of ornithine decarboxylase, was not altered considerably suggesting that embryogenesis and development can proceed normally upon removal of the inhibitors. Analysis of cell cultures in exponential growth phase revealed some differences in PA titers between cell lines. For an IR54 cell line capable of plant regeneration from protoplasts ratios of free to conjugated PAs were 5:1, 6:1, and 3:1 for Put, Spd, and Spm, respectively; the ratio of total Put / Spd + Spm was 0.68. As cells of this line approached senescence the ratio of Put / Spd + Spm increased to 1.5, although the ratio of free to conjugated Put did not change signficantly. An IR52 cell line uniquely effective as a feeder culture for inducing protoplast growth had an elevated titer of free Put, as is evident from the 14:1 bound to free Put ratio and the ratio of total Put / Spd + Spm of 3.4. The role of PA metabolism in cells that function as feeders for protoplast culture will be discussed.

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HYDROXY CINNAMOYL PUTRESCINES DURING IN VITRO DIFFERENTIATION OF INTERNODE EXPLANTS FROM SHORT-DAY, LONG-DAY AND DAY-NEUTRAL NICOTIANA.

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Internodes cut from mature tobacco plants were cultured on Agar medium for 40 days. Explants from day-neutral N. tabacum var. Xanthi developed flowers, while N. tabacum Paraguay Mammoth (short-day) and N. silvestris (long-day grew vegetative shoots only. Feruloylputrescine (FP) content peaked around day 10 after transplanting, and was mainly located in cortex tissue. Caffeoylputrescine (CP) content increased after day 14, and reached a maximum around day 31, mostly in flower buds of N. t. Xanthi explants. Both FP and CP showed similar patterns in all explants, irrespective of cultivar and light regime. The results show that FP and CP are present in different times of development. Hydroxycinnamoyl putrescine pattern is independent of explant source.

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ENDOGENOUS SPERMIDINE-BOUND PROTEINS IN YOUNG AND MATURE TOBACCO OVARIES AND LEAVES

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We have previously reported *in vitro* binding of spermidine (Spd) to a specific protein (about 18kD) in thin-layer tobacco tissue cultures and suggested that polyamines may play a role in plant growth and development through such binding. We now report the existence of Spd-bound proteins in young tobacco ovaries and leaves. These proteins were extracted from frozen tissue of young and mature tobacco ovaries and leaves in a protein extraction buffer followed by $(NH_4)_2SO_4$ cuts. The resulting proteins, after solubilization in NaOH, were hydrolyzed to release the bound Spd which was dansylated, separated by TLC and measured fluorimetrically. The amount of Spd bound to proteins in both kinds of tissue is significantly higher in young than in mature tissues. The bound Spd in the 50% $(NH_4)_2SO_4$ cut is more than 4 x higher in young than in mature ovaries. Further purification of the Spd-bound proteins is being carried out to understand the nature of the binding and its possible significance for cell division.

(Aided by a grant from NSF to AWG)

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POLYAMINE METABOLISM IN TOBACCO IN VITRO FLOWER SYSTEM

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Epidermal strips (also called thin cell layers or TCLs) from the peduncles of tobacco flowers differentiate into flower buds when cultured in a flower inducing medium (FIM) containing equimolar amounts of auxin and cytokinin. Addition of spermidine (1.0 mM) to FIM doubles the number of flower buds in thin cell layers of 'Samsun' tobacco. TCLs grown on a medium containing a 10:1 cytokinin/auxin balance produce only vegetative buds or shoots. Spermidine and spermine addition can stimulate floral bud formation in explants grown in this shoot-inducing medium (SIM). Exogenous spermine added at 0.5-5.0 mM to SIM induces floral bud formation to the same extent as that obtained in FIM. Addition of 0.5 mM difluoromethylornithine (DFMO), an inhibitor of putrescine biosynthesis, inhibits formation of floral buds but promotes vegetative bud formation in 'Samsun' TCLs. Difluoromethylarginine (DFMA) exerts a similar effect as DFMO but of lesser magnitude. α-Aminooxyphenylpropionic acid (AOPP), an inhibitor of phenylalanine ammonia lyase (PAL), causes a 76% reduction of flower bud formation, with no significant effect on the number of vegetative shoots formed. Caffeoylputrescine is not detectable in freshly excised epidermal peels, but accumulates with the onset of floral differentiation. In parallel, free putrescine decreases by the same magnitude. These results suggest that bound polyamines such as phenolic conjugates may be necessary for flower bud differentiation *in vitro*.

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TRANSPORT OF ^{14}C-IAA AND ^{14}C-ACC WITHIN FLORAL ORGANS OF IPOMOEA NIL

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The transport of ^{14}C-IAA & ^{14}C-ACC from agarose donor blocks applied to I. nil filaments & their recovery as ^{14}C-accumulation into floral organs was examined. The accumulation of the isotopes in the corolla tissue was greater when ^{14}C-ACC was applied than ^{14}C-IAA in intact & isolated flower buds (21 h before anthesis). Greater levels of the isotopes accumulated in the pistil, with minimal levels in receptacle and calyx tissues from isolated buds. With intact buds, greater levels of the isotopes were recovered in pistil, calyx & receptacle tissues. This study provides further evidence for the role of the filaments as transport vectors for IAA & ACC for the production of ethylene.

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CYANIDE ACCUMULATION IN DEVELOPING FRUITS

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Cellular and biochemical aspects of cyanogenic glucosides are well understood. This is a contribution to their physiology: Cyanide content of developing fruits was determined in Costa Rican wild lima beans (*Phaseolus lunatus*), oil flax (*Linum usitatissimum*) and bitter almonds (*Prunus amygdalus*). The bean and the almond fruit started accumulating HCN shortly after anthesis and stopped before fruit maturity. In contrast, the flax inflorescence contained more HCN than the mature fruit. The bean contained at all times the monoglucosides linamarin and lotaustralin. The almond and flax contained, at anthesis, the monoglucosides prunasin, and linamarin and lotaustralin, respectively, and, at maturity, the corresponding diglucosides.

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ENZYMATIC REGULATION OF SUCROSE CONCENTRATION IN MUSKMELON FRUIT

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Four enzymes involved in sucrose metabolism were studied during muskmelon fruit development in "sweet" and "non-sweet" genotypes. Sucrose phosphate synthase (SPS) activities increased from 7 $\mu mol \cdot h^{-1} \cdot (g\ fr\ wt)^{-1}$ early in fruit development to an average of 28 $\mu mol \cdot h^{-1} \cdot (g\ fr\ wt)^{-1}$ in mature fruit of sweet genotypes. In fruit of the non-sweet genotype, SPS activities increased to only 10 $\mu mol \cdot h^{-1} \cdot (g\ fr\ wt)^{-1}$. Acid invertase activities decreased in fruit of all genotypes during fruit development. Neutral invertase and sucrose synthase activities were low and did not change during fruit development. The data suggest that sucrose is synthesized within the fruit itself via SPS and that until the capacity to synthesize sucrose exceeds sucrose degradation, sucrose accumulation can not occur. Genotypic differences in final fruit sucrose concentration appear to be the result of differences in fruit SPS activities.

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PURIFICATION OF AN ETHYLENE ELICITOR FROM TOMATO FRUIT CELL WALLS

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An elicitor of ethylene production has been isolated from mature green tomato fruit pericarp pectin. Crude elicitor treated with Staphylococcus aureus strain V8 protease for 15 min at 37°C or with 2 N TFA for 1 h at 120°C was not active, suggesting that both protein and carbohydrate moieties are necessary for activity. Boiling the crude preparation did not destroy activity. Purification of the elicitor was accomplished using DEAE- and CM-Sephadex, BioGel P-100, and C_{18} HPLC. The active material was analyzed for amino acid and carbohydrate composition.

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ACETALDEHYDE REGULATION OF GLUCONEOGENESIS IN TOMATO FRUITS

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Background results revealed that applied acetaldehyde (AA) led in fruit, including tomatoes, to an increase in total and reducing sugars. In the present work we test the hypothesis that these changes may be attributed to gluconeogenesis. This view is supported in part by the results showing that in pericarp discs, from various tomato varieties, the AA-induced increase in sugars was accompanied by: a. a corresponding reduction in organic acids b. recovery in the sugar fraction, of the label from applied ^{14}C malate, and c. arrest of these processes by the gluconeogenesis inhibitor 2,5-anhydro-mannitol.

Interestingly, some gluconeogenesis was found in normally ripening tomatoes. AA appeared to stimulate the process. Additional objectives will be to elucidate the key enzymes which catalyze gluconeogenesis and whether AA mediate their activity or alternatively synthesis.

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THE RIPENING OF DISKS CUT FROM TOMATO PERICARP

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Small disks of tissue cut from the pericarp of mature green tomatoes progress through a sequence of developmental changes that are characteristic of fruit ripening. A system for handling tomato disks, using 24-well microtiter plates, allows easy measurement of ethylene biosynthesis, respiration, and color change in individual disks, application of treatments, and selection of disks for destructive analyses. Over a period of several days, color of disks changes from green to full red, ethylene production increases, and tissue firmness declines. Changes in cell wall polysaccharide constituents and polymer hydrolases (including polygalacturonase) have been followed in conjunction with tissue softening. Ripening of disks seems an accurate model of ripening in intact fruit. This system should be an excellent tool for the study of metabolic aspects of ripening, especially for application of tracers and metabolic intermediates.

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A GC-MS METHOD USING $^{13}C6$-GLUCOSE LABEL FOR EVALUATING SYNTHESIS OF CELL WALL POLYSACCHARIDES IN RIPENING TOMATO PERICARP DISKS

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Recent work by various investigators has shown that there is biosynthesis of cell wall polysaccharides in some types of fruit continuing into the final stages of ripening. It has been suggested that this newly synthesized polysaccharide may contribute to the ripening-related softening of these fruits. Because of this it is of interest to understand the nature of these biosynthetic events. We have recently developed a GC-MS technique which allows us to evaluate the incorporation of $^{13}C6$-glucose into the polysaccharides of ripening disks cut from the pericarp of mature green tomatoes. We have followed metabolism and incorporation of the labelled glucose into several polysaccharide types. Labelled glucose was provided to disks at various stages of ripeness so as to determine changes in uptake/biosynthetic capacity that accompany ripening.

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CHANGES IN IAA AND ABA CONTENT IN DEVELOPING TOMATO FRUITS

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IAA and ABA levels in developing tomato fruits were determined from 10 days before to 30 days after anthesis. IAA was measured fluorometrically and ABA was by GLC with electron captured detector. The lowest concentration of IAA was found in the flowers at anthesis and that of ABA was in those at 6 days after anthesis. Thereafter both IAA and ABA in fruits showed marked increase. IAA in flowers existed more in stamens and less in pistils. ABA in flowers was high in pistils and low in stamens. IAA levels in fruits(ca.40 mm in diameter) of 30 days after anthesis were high in locules and low in pericarps, whereas conspicuously high ABA level was found in the axis of ovaries.

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SPECIFIC CHANGES IN MEMBRANE POLAR LIPID FATTY ACID COMPOSITION COINCIDES WITH INITIATION OF FRUIT RIPENING AND CHANGES IN FREEZING STRESS RESISTANCE OF LEAVES OF CRANBERRY

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Fruit ripening, freezing injury and cold acclimation are thought to involve a membrane response. Cranberry fruits and leaves were sampled over 3 seasons. Polar lipids were extracted from the tissue and fatty acid composition was determined. The most pronounced changes were, an increase in 18:2 and a decrease in 18:0 and 18:1. Changes in the LR (lipid ratio), (18:2)/(18:0+18:1), was calculated. Significant increase in LR coincides with the initiation of fruit ripening and with the increase in FSR of the leaves. Similar changes in LR were obtained in artificially ripened fruits. A decrease in LR coincided with a decrease in FSR. Artificially cold acclimated leaves showed similar changes in LR. Our results suggest that the changes in lipid environment (chemical/physical) play an important role in both fruit ripening and the seasonal changes in FSR in leaves. FSR= Freezing Stress Resistance.

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REGULATION OF GENE EXPRESSION DURING SENESCENCE

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Here we report progress on initial studies designed to identify ultimately genes regulated during senescence in wheat and bean. In this preliminary report we have compared differences in electrophoretic patterns of proteins extracted from excised leaf tissue aged for various periods of time in the dark. We will exhibit electrophoretograms demonstrating proteins of interest to the goal of this study.

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EFFECTS OF THERMAL POWER PLANT EFFLUENTS ON FORMATION AND SENESCENCE OF REPRODUCTIVE PARTS OF ANAGALLIS ARVENSIS L.

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Oxides of sulphur, nitrogen and carbon and particulates are the major air pollutants emitted in huge amounts by the Thermal Power Plant Complex of Kasimpur(Aligarh,UP,India)running on 3192 MT of coal/day.These effluents significantly affect reproductive phase of Anagallis arvensis L. Samples of 10 plants each were randomly collected at monthly intervals at seedling to mature stage from 0.5,2,6,12 and 20 km leeward from the power plant complex. Bud formation and flowering were delayed in the population thriving at 0.5 km from the pollution source. At a 2 month old stage, 60% of the population showed a decline in bud formation in the vicinity of the source compared to a heavy bud emergence in the whole population thriving 20 km away from it. Bud formation, flowering fruit set and seed set showed a correlation with multiple growth factors viz productivity, shoot length and distance from the source.

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POD DEVELOPMENT AND DEPODDING PRODUCE PARALLEL EFFECTS ON XYLEM SAP CYTOKININ LEVELS AND MONOCARPIC LEAF SENESCENCE IN SOYBEAN

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Depodding at full pod extension (prepodfill) prevents the rapid leaf yellowing and death of the soybean plant, whereas pod removal in late podfill does not. Xylem sap was collected from rootstocks under pressure over 50 min, and after purification, the sap CKs were measured by radioimmunoassay. The major CKs (DZR, ZR, DZ and Z) drop from 229 nM to 15 during pod extension. Removal of pods reaching full extension causes a large increase in DZR and ZR levels, less increase in Z and no increase in DZ, DZMP or the O-glucosides. Depodding at the late podfill does not increase the CKs significantly. CKs change independently in response to senescence and pod removal suggesting differences in their metabolism and perhaps their functions. A decline in CK production by the roots and in CK flux into the shoot system appears to be an important factor in monocarpic senescence of soybean.

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CULM BRITTLENESS AND CELL WALL COMPONENTS OF BRITTLE BARLEY CULM

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The fourth internode of four brittle and two non-brittle barley (Hordeum vulgare) strains were used for physical and chemical studies of culm strength. Maximum bending stress, at which the culms was broken, was 190 ± 34 g/mm^2 for brittle and 498 ± 38 g/mm^2 for non-brittle strains. The area of cell wall per unit cell area in each tissue was significantly correlated with the maximum bending stress ($r=0.93$ for epidermis, 0.90 for sclerenchyma and 0.84 for parenchyma). Cell walls of brittle culms had 6 to 64% as much cellulose content of the cell walls ($r=0.93$), but not with the contents of non-cellulose compounds. The lower cellulose content of the brittle culm is one of the main causes of the brittleness.

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THE INFLUENCE OF EXISTING TILLERS ON ELONGATION OF LEAF AND AXILLARY BUDS IN TALL FESCUE

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Rapid leaf elongation in tall fescue is accompanied by a reduction in tillering. Leaf and tiller development were monitored in three populations differing in leaf elongation rate and tiller production. Young tillers were removed from treatment plants shortly after they appeared above the ligule of their subtending leaf. Tiller removal had little effect on leaf elongation rate but reduced the duration of elongation. Duration of elongation in the expanding leaf controlled the rate of new leaf and tiller appearance. Cessation of elongation was associated with the appearance of the tiller found at the same node as the expanding leaf as well as initiation of elongation in the leaf located two nodes above. Increased tiller density had no effect on the triggering of leaf elongation but did delay new tiller appearance. A clear synchrony of initiation events was displayed suggesting a high level of communication among leaves and tillers on a plant.

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ACCUMULATION OF RESERVES DURING ONOCLEA SPORE DEVELOPMENT

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Onoclea spores at maturity contain on a dry weight basis 4 to 6% sucrose and 20 to 30% each of proteins and lipids. Initial studies on establishment of these reserves dealt with determining the order of deposition of the reserves during spore development. Spores were collected biweekly from the time of sporophyll emergence to the time of dehydration & were analyzed for carbohydrate and lipid content. Sucrose accumulated throughout the developmental period with ca. 4,5% of the dry weight attained by the time of dehydration. Starch accumulated during the first 4 weeks after sporophyll emergence but declined thereafter to the low level characteristic of mature spores. Total lipids accumulated to ca. 30% of the dry weight during the first 4 weeks also and then remained constant. Classes of simple lipids present included tri-, di-, and monoacylglycerols, free fatty acids, and cholesterol esters as revealed by TLC. Studies are in progress to characterize and to quantify changes in storage proteins during spore development.

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Ca^{2+} REGULATION OF PEA PLASTID TRANSLATION AND PROTEOLYSIS

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Inhibition of protein synthesis in intact pea chloroplasts by Ca^{2+} ions is enhanced by 5 μM A23187, and is not reversed by EGTA added later. Together with a 3 to 5 minute delay in onset of inhibition, and partial reversal by calmodulin antagonists, a surprisingly complex mechanism is suggested. Pulse-chase experiments show some extra proteolysis of newly produced chloroplast proteins induced by Ca^{2+}, which might account for much of the inhibition. Ca^{2+} did not inhibit translation by lysed chloroplasts, unless they had been pre-incubated with 0.1 mM Ca^{2+} for 20 minutes and then lysed. However, sensitivity to Ca^{2+} was restored to chloroplast lysate translation either by adding 20-100 μM CHAPS, or by adding a soluble extract from other chloroplasts lysed following preincubation with Ca^{2+} while intact. A working hypothesis to explain these data is that two Ca^{2+} effects occur: an activation step at the envelope, releasing a macromolecular Ca^{2+}-dependent inhibitor, or protease, into the stroma. The factor may not escape from envelope vesicles in the lysate unless a detergent (CHAPS) is added.

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IN VITRO SYNTHESIS AND PURIFICATION OF UDP-[^{14}C]GALACTURONATE

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Pectins comprise a major component of the cell wall and much research has focused on degradation of pectins during ripening and senescence. However, little research has been conducted on pectin synthesis, partly due to a lack of commercial availability of UDP-[^{14}C]galacturonic acid for use as a substrate in assaying galacturonan synthase. We report on the modification and integration of several procedures to synthesize UDP-[^{14}C]galacturonic acid from commercially available UDP-[^{14}C]glucuronic acid. A microsomal pellet containing UDP-D-glucuronate-4-epimerase (E.C. 5.1.3.6) was extracted from 5-day-old mung bean hypocotyls (Phaseolus aureus) and radish roots (Raphanus sativus L.) by ultracentrifugation at 30,000 rpm for 50 min. The UDP-[^{14}C]galacturonic acid produced was separated from remaining UDP-[^{14}C]glucuronic acid and other products by electrophoresis in pyridine:acetate:H_2O on silica gel. Spots were detected by autoradiography, eluted with 80% ethanol, and purified using anion exchange chromatography.

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THE MAJOR STORAGE PROTEINS OF TUBERS OF HYDRILLA VERTICILLATA

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Major proteins were characterized from tubers of both monoecious and dioecious Hydrilla verticillata. These proteins comprised approximately 30% of the total protein in the tuber, and 15% of the total nitrogen. Their molecular masses were ca 58 kD with isoelectric points near pH 4.2. Two-dimensional electrophoresis followed by Western blotting and treatment with antibodies against the major proteins showed three polypeptides in the monoecious extract, and two in that from the dioecious. Tubers of related aquatic macrophytes, the Potamogetonaceae, also had major proteins which were cross-reactive with the antibodies against the tuber proteins of H. verticillata. In tubers of P. pectinatus, there were at least 6 cross-reactive proteins when two dimensional electrophoresis and Western blotting were carried out. The N terminal amino acid sequences of the most abundant polypeptides from the two varieties of H. verticillata will be reported.

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EFFECT OF TUBER AGE ON CYTOCHROME-MEDIATED AND ALTERNATE RESPIRATION OF TISSUE FROM SPROUTING POTATO TUBERS

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The physiological age of potato seed-tubers influences growth capacity. Seed-tubers stored for prolonged periods sprout with reduced vigor compared with younger seed-tubers. To determine if age-related reductions in vigor are associated with less efficient respiratory activity, the contribution of the different modes of respiration was measured in tissue from 7 and 19-mo-old tubers at 6 d intervals over 18 days of sprouting. Tuber age and time had no effect on cyt-mediated respiration, which averaged 18 nmol O_2/min/g.f.wt over the study period. As a percentage of total respiration, residual respiration was relatively constant (26%) in both ages of tissue during sprouting. In younger tuber tissue, alt respiration decreased from 9 to 1% of total by day 12. Over the same period, the proportion of alt respiration in older tissue increased from 14 to 28%, and this occurred with a concomitant decrease in cyt-mediated respiration. During sprouting, older tubers appear to have a less efficient energy-metabolizing pathway.

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CHARACTERIZATION OF PLANT GROWTH FROM AGED POTATO SEED-TUBERS AND AMELIORATION OF AGE-INDUCED EFFECTS WITH AUXIN

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Plant growth from single-eye cores from potato tubers stored for 5 to 18 months was compared. Loss in apical dominance was apparent with advanced age. On a per-core basis, the amount of plant dry wt was equal for the two ages at 30 d from planting. However, individual plants from older cores displayed reduced shoot, root and leaf dry wts, leaf area, and leaf no. These effects reflected altered dry-matter partitioning and contributed to an overall change in plant morphology with advanced age. To determine the role of auxin in the age-induced loss of vigor, cores were treated with 0, 50, 100 and 150 ppm NAA prior to planting. NAA reduced growth from younger cores. On older cores, NAA stimulated root growth, restored apical dominance, decreased leaf no./plant, and increased avg leaf area/leaf. In short, NAA altered plant morphology from older cores to more closely resemble that from younger cores. Even though auxin significantly influenced plant form, an auxin imbalance is not solely responsible for age-reduced vigor of potato.

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PROTEIN SYNTHESIS INHIBITOR FROM POTATO TUBER.

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A protein fraction capable of inhibit in vitro protein synthesis was found in potato tubers in fresh and wounded tissue. Inhibitor activity from fresh tissue decays with wounding. Inhibition activity was detected adsorbed to ribosomal fraction and cytosol of potato tuber tissue by a partially reconstituted in vitro system from potato tuber and wheat germ. Adsorbed ribosomal fraction was more suitable of purification. This fraction was washed from ribosomes with 0.3M KCl, concentrated with ammonium sulphate precipitation and purified through sephadex G100 and sephadex G-75 columns chromatography. After 61 fold purification adsorbed protein fraction can inhibits germination of maize, wheat and sesame seeds, as well as ^{3}H-leucine incorporation into protein by imbibed maize embryos. Inhibition activity was lost by temperature, alkali and protease-K hydrolysis. Preliminar analysis could not show presence of reductor sugars. Physiological role of this inhibitor in relation to rest and active tissue remains to be studied.

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UDP-[^{14}C]GLUCOSE-LABELABLE POLYPEPTIDES FROM PEA: POSSIBLE COMPONENTS OF GLUCAN SYNTHASE I ACTIVITY

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A membrane-bound polypeptide doublet of about 40 kD can be rapidly labeled with UDP-[^{14}C]glucose under the assay conditions for glucan synthase I (GS-I). Label seems covalently bound, and chases when unlabeled UDPG is added; it might represent a covalent intermediate in polysaccharide synthesis. Labeling and GS-I activity show several common features: they co-sediment with Golgi membranes in sucrose gradients; they depend similarly on Mg^{2+} or Mn^{2+} (not Ca^{2+}); they decrease dramatically from stem apex to base, and are higher in epidermis than internal tissue; they show similar sensitivities to several inhibitors. But the doublet still labels after polysaccharide-synthesizing activity has been destroyed by Triton X-100. The doublet polypeptides might be glucosyl tranferases whose ability to transfer glucose units to a glucan chain is detergent-sensitive, but to accept glucose from UDPG is not; or they might be detergent-insensitive primary glucose acceptors, from which a distinct, detergent-sensitive transferase(s) move(s) these units to glucan chains.

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DIFFERENCES IN THE ACCUMULATION OF VEGETATIVE STORAGE PROTEIN IN TISSUES OF VIGNA AND GLYCINE SPECIES

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Vegetative storage proteins (VSPs) have been identified in the leaf paraveinal mesophyll tissue of soybean and other Glycine species. VSP accumulates during vegetative growth, but the highest levels are obtained in plants deprived of reproductive sinks. Some species of the Phaseolinae also accumulate proteins during sink deprivation, and in some cases such as in Vigna subterranea and V. acontifolia, the accumulated polypeptides are immunologically similar to Glycine VSPs. In contrast to the Glycine species, the Vigna species accumulate higher levels of VSP in the stems, petioles, and pulvinus than in the leaves. In V. acontifolia, the level of VSP in the leaf blade decreases as the leaf ages and the level in the petiole, the major VSP storage tissue, increases. Both Vigna species are minor crop plants that are normally grown in water stressed environments. VSP may be a temporary nitrogen store where nitrogen accumulates during wet periods for later pod fill under dry conditions.

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TOOLS TO STUDY THE POST-TRANSLATIONAL MODIFICATION OF WGA

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Wheat germ agglutinin (WGA) is synthesized during embryogeny and accumulates in morphologically distinct cell layers of several embryonic organs. In hexaploid wheat, each diploid genome (A, B & D) directs the synthesis of its own unique isolectin (WGA isolectins A, B & D). In addition to the previously characterized clone for isolectin B, WGA-B, we have now isolated and determined the nucleotide sequences of two full length cDNA clones, WGA-A and WGA-D, encoding isolectins A and D respectively. WGA-A and WGA-D are >90% homologous to WGA-B at the nucleotide level (>93% in the coding region) while WGA-A and WGA-D share >94% homology between themselves (>95% in the coding region). These clones helped us to determine that WGA is synthesized as a preproprotein with a hydrophobic signal sequence and a glycosylated, 15 amino acid C-terminal propeptide. During transport to the protein bodies/vacuoles, the signal sequence is co-translationally cleaved and the glycopeptide is then removed from pro-WGA to produce the mature lectin. To begin to study the post-translational modification of WGA, a synthetic peptide corresponding to the propeptide of WGA was made (Carb-15) and an antiserum against Carb-15 was raised in rabbits. Anti-Carb-15 reacted only with pro-WGA when Western blots of WGA preparations were probed with anti-Carb-15 and was also specific for pro-WGA in ELISA. Finally, anti-Carb-15 has been used to quantitate pro-WGA in developing wheat embryos, both at different developmental stages and after ABA treatment.

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ANATOMICAL CHANGES AND IMMUNOLOCALIZATION OF CELLULASE DURING ABSCISSION AS OBSERVED ON NITROCELLULOSE TISSUE PRINTS

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Anatomical tissue imprints on nitrocellulose (NC) membranes provide a low resolution three dimensional image of the tissue when viewed with side illumination. Changes in the tissue integrity taking place in the abscission layer as the process of abscission proceeds can be easily observed in either longitudinal or serial cross sections through the abscission zone followed by printing on NC membranes. A fundamental event in abscission is the breakdown of cell wall material in a discrete zone of cells. In bean leaf abscission the dissolution of cell walls has been attributed to the appearance of a form of cellulase with an isoelectric point of 9.5. Antibodies specific for this enzymes has been used to study the immunolocalization of 9.5 cellulase in the distal abscission zone of P. vulgaris using tissue printing on NC. It was found that 9.5 cellulase is localized in the abscission layer and in the vascular tissue of the adjacent pulvinus and petiole. No label was observed in non-abscising tissue or pre-immune controls. These results confirm previous biochemical studies and overcame the shortcomings of previous immunolocalization studies where the enzyme distribution in the stele was masked by non-specific staining.

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EFFECTS OF CARBON DIOXIDE AND ETHYLENE AT THE ULTRASTRUCTURAL LEVEL OF ABSCISSION CELLS. Valdovinos, J.G., S.J. Lieberman and T.E. Jensen, Dept. of Biol. Sci., Herbert H. Lehman College, CUNY, Bronx, N.Y. 10468.

A study of the structure of abscission cells of tobacco flower pedicels treated with air, ethylene (ETH), carbon dioxide (CO_2), and ETH plus CO_2 indicated the following: CO_2 treatment suppressed ETH - increased rough endoplasmic reticula. With both CO_2 and ETH + CO_2 treatments, the following changes were observed: mitochondria appeared less electron dense; greater numbers of Golgi vesicles were present; chloroplasts contained greater numbers of starch granules as well as starch granules of a larger size and interval spacing between thylakoids was increased; vesicles associated with cell walls were increased in number.

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LEAF MOVEMENTS IN PHASEOLUS VULGARIS L. AFTER REGENERATION OF PULVINAR TISSUE CONSECUTIVE TO A PARTIAL EXCISION.

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Primary leaves of bean plants (Phaseolus vulgaris L.) show circadian and superimposed ultradian movements. The question arises whether all of the pulvinar cells involved in movements display simultaneously both rhythms or each rhythm operates in two cellular types located in different areas of pulvinus. Removing of a part of the lower (or upper) half of the pulvinus was followed after 3 days by complete regeneration of the excised part. Such ability could be related to a high content in cytokinins and zeatin-riboside in the pulvinus compared to that of the petiole. Movements monitored after regeneration showed some modifications in period and/or amplitude. A comparison of original and neoformed cells has been carried out. Some significant differences could be seen in fresh and dry weight, water, K^+ and malate content and vacuolar pH.

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QUANTITATION OF PEROXIDASE ACTIVITY DURING GRASS LEAF DEVELOPMENT

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Peroxidase activity may limit cell wall extensibility by causing covalent bond formation, IAA oxidation, or lignification. Change in localization and specific activity of peroxidase during cell elongation and secondary cell wall deposition were determined in tall fescue leaf blades. Anatomical studies indicated that, except for protoxylem, no significant accumulation of secondary cell wall or lignin occurs in tissues before cessation of epidermal cell elongation. Peroxidase activity, localized histochemically, was present in both xylem and phloem in the region of active cell division, in epidermal and bundle sheath tissues during early cell elongation, and in virtually all tissues before cessation of epidermal cell elongation. In grass leaves, cell maturity increases with distance from the leaf base. Specific activity of cell wall peroxidase decreased with distance from the leaf base within the region of epidermal cell elongation, then increased with distance within the region of secondary cell wall deposition. This pattern of peroxidase activity was correlated positively with dry weight per unit leaf area, a measure of cell wall material, regardless of leaf elongation rate. These results suggest that cell wall peroxidase activity is related to both cell differentiation and accumulation of cell wall material in tall fescue leaf blades.

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ANALYSIS OF LEAF DEVELOPMENT IN THE HETEROPHYLLOUS AQUATIC PLANT HIPPURIS VULGARIS

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Heterophyllous aquatic plants are characterized by the production of distinctly different leaf forms depending on whether their shoot apices are above or below the water surface, and this phenomenon is thought to be regulated by abscisic acid(ABA). The objective of the present study was to characterize the timing and location of specific developmental changes that result in the different final leaf forms. We addressed this objective with marking and structural studies in conjunction with exogenous ABA treatments to control leaf development. During the first 20 plastochrons (up to 13 mm in length) leaves are plastic and can develop either aerial or submerged characteristics depending on the presence or absence of ABA. These and other results indicate that 1) commitment of a region of cells as aerial or submerged type occurs at the cellular or local level, as opposed to the whole leaf level, and 2) final leaf determination occurs autonomously and does not depend on the amount of time spent at the apex.

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DO SHORT CHAIN FATTY ACIDS HAVE A REGULATORY ROLE IN APPLE?

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Short chain (<12C) fatty acids (SCFA) are found in various plant tissues. There have been reports in the last 2-3 decades that these substances are inhibitory in certain biological systems and thus may have an important regulatory role. We have examined some of these substances in apple buds and seeds. The inhibitory properties of normal saturated SCFA in an in vitro apple bud explant test was found to be chain length dependent, maximum inhibition being obtained with chain lengths of C_8-C_{10}. We have also examined changes in one of these SCFA (putative decanoic acid, C_{10}) in apple seeds of 2 cultivars which have decidedly different chilling requirements. Decanoic acid decreased in both cultivars as stratification proceeded, reaching relatively low concentrations by the time that stratification was complete in each cultivar. These positive correlations suggest that SCFA could play a regulatory role. Further investigations are underway.

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THE ROLE OF ENVIRONMENTAL FACTORS IN THE FORMATION OF RESERVES IN A PERENNIAL WEED (Euphorbia esula L.)

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We have examined storage metabolism in the roots of the noxious perennial leafy spurge. In winter soluble carbohycrates increase and predominate quantitatively as storage reserves forming a primary source of reserve energy. However, it appears that nitrogen is as significant qualitatively with regard to overall storage strategy, with nitrate, free amino acids and soluble protein all playing important roles. Two approaches have been utilized to examine the role that the environment plays in the seasonal variation in storage reserves. Changes and contents of carbohydrates were little affected by defoliation treatments; however, accumulation of free amino acids, and more significantly, soluble protein was reduced. Associated with the latter were distinct changes in electrophoretic profiles. Both photoperiod and day/night temperature may play important roles in the induction of reserves for winter storage.

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LIGHT-DARK REGULATION OF SULFATE ASSIMILATION IN LEMNA MINOR L. IN THE PRESENCE OF O-ACETYL-L-SERINE.

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The effect of light removal and addition of O-acetyl-l-serine (OAS) (0.5 mM) on sulfate assimilation in Lemna minor L. was analysed by measuring the extractable activity of adenosine 5'-phosphosulfate sulfotransferase (APSSTase) and the in vivo incorporation of $^{35}SO_4^{2-}$. After removal of light APSSTase activity decreased to 10% within 24 h in the absence and to 50% in the presence of OAS. Within 24 h total $^{35}SO_4^{2-}$ uptake decreased to 60% without and increased to 130% with OAS compared to light controls. The incorporation of ^{35}S into cysteine increased 2 times without and 15 times with OAS, labelling of glutathione decreased to 65% and increased to 140%, the one of the protein fraction decreased to 30% and to 20% of the light control in the absence and presence of OAS. Our results indicate that OAS has a regulatory function on the assimilation of sulfate and that protein synthesis is inhibited in the dark.

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KINETIC RESPONSES OF GROWTH, AUXIN TRANSPORT, AND ETHYLENE EVOLUTION BY 'ALASKA' PEA TO MECHANICAL STRESS.

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Thigmic (contact) mechanical stress inhibits epicotyl elongation as well as basipetal auxin transport by dark-grown seedlings of *Pisum sativum* L. cv. Alaska. On the other hand, low-frequency vibric (vibrational) stress similar to that occurring on board orbiting spacecraft mildly stimulates growth of pea seedlings following initial suppression of growth rate. Results of experiments in progress to be reported include real-time kinetic responses of growth rate, velocity and polarity of auxin transport, ethylene evolution, and effects of ethylene antagonists on these plant responses to vibric stress.

Research supported in part by NASA grant NAG 100018.

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MECHANICAL STRESS-INDUCED CHANGES IN CELL WALL EXTENSIBILITY COMPLIANCES

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Physical disturbance (i.e. mechanical stress) reduced elongation growth of dark-grown soybean and sunflower hypocotyls 40 to 50% in a 24-h period. Measurable growth inhibition is apparent within 1 h after stress application. It was hypothesized that stress-induced reduction in cell wall extensibility may be partially responsible for the observed growth inhibition. Instron-type examination of cell wall extensibility compliances revealed that extensibility apparently increased, rather than decreased, in stressed hypocotyls. Thus, stress-induced growth reductions are not likely to result from an increase in cell wall strength.

Supported by NASA Grant NAG 100018

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ASYMMETRIC AUXIN REDISTRIBUTION ACROSS CAPS OF GRAVISTIMULATED MAIZE ROOTS IS ESSENTIAL FOR GRAVICURVATURE

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A recent model for root gravitropsim (Sci. Am. 254:112) implicates the cap as the site of initial auxin redistribution. Kinetics of gravi-induced (GI) polar auxin movement across the cap parallel kinetics of gravicurvature (GC). Pretreatment with auxin transport inhibitors (NPA, PBA) prevented polar auxin movement across caps and eliminated GC. Polar auxin movement also occurred across caps gravistimulated (GS) after isolation from the root and was inhibited by NPA and PBA. Since calcium (Ca^{2+}) has been implicated in polar transport mechanism, we examined the Ca^{2+}-dependence of polar auxin transport across the caps of GS roots. EGTA inhibition of asymmetric auxin movement was reversed by Ca^{2+}. The data suggest Ca^{2+} may be essential for GI polar auxin movement across root caps.

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TRANSPORT OF STELE-ASSOCIATED IAA IN GRAVISTIMULATED ROOTS OF MAIZE

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We tested the redistribution of label from a pulse of ^{3}HIAA applied to the cut surface of 5 mm apical root segments. We determined the amount of label in the stele, meristematic region (MR), root cap, and cortical tissue on the lower and upper sides of gravistimulated vs control roots. Label entered the stele and moved into the MR prior to accumulating in the elongation zone (EZ). Little label moved into the cap. Gravistimulation did not increase the amount of label moving from the stele, but it increased the amount of label accumulating on the lower side of the EZ. Removal of the cap immediately following gravistimulation rendered the roots insensitive to gravity and prevented gravi-induced asymmetric redistribution of label. The results suggest that a signal originating in the root cap directs auxin redistribution in tissue behind the cap.

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AMYLOPLASTS AS POSSIBLE STATOLITHS IN GRAVITROPIC PROTONEMA OF THE MOSS CERATODON.

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No gravitropic tip growing cell has been characterized in which amyloplasts are likely to function as statoliths. Dark grown protonema of Ceratodon purpureus are negatively gravitropic. Of the 4 distinct zones in the apical cell, only one contains sedimenting amyloplasts. There is a statistically significant movement of amyloplasts towards the lower wall after 15 minutes of gravistimulation; upward curvature is first detected 60-90 minutes after the start of gravistimulation. If protonema are given a single exposure to gravity and then placed on a clinostat, curvature develops after a single 30 min. but not a 20 min. dose. Amyloplasts in the sedimentation zone are displaced by lower g's (4.5g x 20 min.) than are amyloplasts right in the tip (22g x 90 min.). This is the first modern description of a cell that both perceives and responds to gravity that may employ amyloplasts as statoliths.

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MODIFICATION OF PATTERNS OF GRAVITROPIC CURVATURE OF MAIZE ROOTS. II. INTERACTION OF AUXIN AND ETHYLENE

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Time-lapse videography and computer-based video image digitization were used to characterize the kinetics of gravicurvature (GC) of primary roots of maize (*Zea mays* L., Pioneer 3343) pretreated with ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG, 1 μM, 60 min) prior to unilateral application (UA) of IAA. Elongation of vertically-oriented roots is inhibited by IAA conc. greater than 10^{-10} M while pretreatment with 1 μM AVG shifts the auxin sensitivity so that inhibition occurs at conc. greater than 10^{-8} M. UA of IAA (10^{-4}-10^{-7} M, 1 mm^3 agar block) to the upper surface of the elongation zone (EZ) of horizontally-oriented (HO) roots resulted in minor upward GC (9-15°) while application to AVG-pretreated roots resulted in exaggerated upward GC (20-50°). UA of IAA (10^{-4}-10^{-7} M) to the lower surface of the EZ of a HO roots decreased the presentation time for GC; no significant difference in the kinetics of GC was observed in IAA-treated roots which received or did not receive AVG pretreatment. UA of IAA (10^{-10}-10^{-11} M) to the upper surface of the EZ did not significantly alter the early kinetics of GC; in AVG-pretreated roots the application of low concentration of IAA to the upper surface delayed the initiation of GC by 30-40 min. A notable effect of low level IAA treatments was the suppression of both the oscillatory movement and overshoot of vertical orientation observed in control roots.

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MODIFICATION OF PATTERNS OF GRAVITROPIC CURVATURE OF MAIZE ROOTS. I. THE ROLE OF ETHYLENE

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Time-lapse videography and computer-based video image digitization were used to examine the kinetics of gravicurvature (GC) of primary roots of maize (*Zea mays* L., Pioneer 3343) treated with the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG) and the ethylene precursor ACC. In control roots, approx. 20-30% of the roots exhibit negative GC (9-15°, duration of 15-30 min) within 4-12 min of horizontal orientation (HO); this is followed by positive GC (85-110°) with rapidly-damped oscillatory movement. In 70-80% of the roots the initial response is positive GC within 8-15 min of HO; roots overshoot vertical orientation (VO) to a maximum of 165°, and undergo sustained oscillatory search for VO. The oscillations are up to 70° either direction from VO. AVG pretreatment of roots (1 μM, 60 min) induces negative GC of 15-30° and delays positive GC for 65-85 min; roots curve 210-270° over the next 12-14 hr then to reverse direction of GC for 180-240°. Application of ACC to the elongation zone (1μM, 1 mm^3 agar block) delays GC for 90-120 min; after initiation of positive GC, the roots curve 170-220° over the next 12-16 hr then reverse direction of GC for 110-140°. The data suggest that sub- or supra-optimal conc. of ethylene alters the graviresponsiveness of roots.

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MAIZE ROOT TIPS CONTAIN AN mRNA SPECIES WITH HOMOLOGY TO THE CONSERVED CATALYTIC DOMAIN OF PROTEIN KINASES

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Protein phosphorylation by various kinases is an important component of eukaryotic signal transduction systems. In order to study the role of protein kinases in root gravitropism, maize cDNA clones homologous to known protein kinases were identified. A maize root tip cDNA library was screened with degenerate oligonucleotide probes encoding conserved regions of animal and yeast protein kinases. The same oligonucleotides were used as sequencing primers to futher characterize hybriding cDNAs. Extensive upstream homology to serine/threonine kinases has been found in at least one cDNA clone. Peptide sequence homology to groups of protein kinases with similar regulatory modes or substrate specificities will be discussed.

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AXIAL FORCES AND NORMAL DISTRIBUTED LOADS IN TWINING STEMS OF MORNING GLORY

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The mechanical nature of the twining habit is investigated. A force balance in the natural coordinate system indicates that, if shear forces are neglected, an axial force within the stem is balanced by a normal distributed load along the line of contact between the supporting pole and the stem. Axial strains due to twining are inferred from a comparison of vine geometry on and off the supporting pole. When removed from the pole, the helical stem forms a coil of smaller radius and larger pitch angle. Stresses are estimated using the geometric strains and a modulus in bending from moment-curvature determinations. Preliminary results indicate that the twining stem sustains a tension of between 200 g and 1000 g balanced by normal loads of 50 g/cm to 260 g/cm. These calculated results are empirically verified by manometric measurements of the pressures exerted by a stem twining around a water-filled balloon.

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DIFFERENTIAL REGULATION OF TWO BARLEY ALEURONE CYSTEINE PROTEINASES BY GIBBERELLIC ACID.

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We have purified and characterized two cysteine proteinases secreted from barley aleurone layers in response to gibberellic acid (GA_3). The hormonal regulation of synthesis of these proteineases was studied. The size of the mature proteins, estimated by SDS-PAGE as 37 and 30 kD (37 kD EP and 30 kD EP respectively), is 11-12 kD smaller than proteins synthesized *in vitro* and immunoprecipitated by the corresponding proteinase-specific antibodies. This suggests the post-translational cleavage of a large pro-sequence *in vivo* as is the case for papain. The proteinases are differentially induced by GA_3 with translatable mRNA for 30 kD EP peaking within approximately 12 hours, while 37 kD EP translatable mRNA peaks at least 36 hours later. The rate of *in vivo* synthesis and secretion of the proteinases in response to GA_3 will be compared to the timecourse of accumulation of corresponding translatable mRNA.

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cDNA CLONES THAT HYBRIDIZE TO RNA WHOSE ABUNDANCE IN TOMATO LEAVES AND STEMS IS RAPIDLY AFFECTED BY GIBBERELLINS

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A cDNA library was constructed using poly(A)$^+$RNA that was extracted from leaves and stems of the dwarf, GA-deficient, *gib-1* mutant of tomato [*Lycopersicon esculatum* (L.) Mill.] (cv. Moneymaker) 4 hrs after spraying with 5 x 10^{-5} M GA_3. Over 100,000 colonies were screened by a differential,dual-labeling method using poly(A)$^+$RNA from 4 hr GA_3 or ddH_2O treated dwarf tomato to identify clones that hybridize to RNA whose abundance changes following treatment. Based this on screen, 16 clones have so far been chosen for further study. Northerns blot analysis reveals that several of these cDNA clones hybridize to poly(A)$^+$RNA that increases in abundance in dwarf tomato leaves and stems within 45 minutes of GA_3 application. In addition, two clones have been shown by preliminary experiments to hybridize to poly(A)$^+$RNA that rapidly decreases in abundance following GA_3 treatment. Further characterization of these clones is in progress.

(Supported by NIH Grant GM40553-01)

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MAPPING OF THE *gai* MUTATION RELATIVE TO RFLPs IN *Arabidopsis*

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The *gai* mutation of *Arabidopsis* results in dwarf plants which are impaired in several responses to exogenous gibberellins (1). Therefore, it is possible that this mutation inactivates a primary step in the pathway by which gibberellin signals plants. As a prelude for attempts to isolate the gene corresponding to the *gai* locus, we have mapped the mutation relative to DNA polymorphisms on the linkage map of Chang et al (2).

1. Koorneef et al. Physiol. Plant. 65:33-39 (1985)
2. Chang et al. Proc. Natl. Acad. Sci. USA 85:6850-6860 (1988)

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THE EFFECTS OF THERMOINDUCTION ON ENDOGENOUS GIBBERELLIN LEVELS IN *THLASPI ARVENSE* L.

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Cold induced stem growth in field pennycress is mediated by a change in the endogenous GA status. In this paper, the effects of thermoinductive treatments on GA levels as measured by GC-MS are reported. Thermoinduction did not result in an increase in the levels of any of the 13-OH GAs native to field pennycress. The levels of GA_1 remained constant (0.4-0.6 ng $plant^{-1}$) in plants harvested prior, during and after the cold treatment. The other 13-OH GAs ($GA_{19, 20, 29, 44, 53}$) accumulated in plants not exposed to cold. In contrast, the GAs lacking a 13-OH ($GA_{9, 15, 24}$) were detected only in thermoinduced plants. GA_9 levels rose to 1.6 ng $plant^{-1}$ 4 d after the cold treatment and were correlated with stem elongation. These results may be an indication that GA_9 rather than GA_1 controls thermoinduced stem elongation in field pennycress.

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COMPARISON OF ^{14}C-GA_{12}-ALDEHYDE METABOLISM IN THERMO- AND NON-INDUCED SHOOT TIPS OF *THLASPI ARVENSE* L.

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The metabolism of exogenous ^{14}C-GA_{12}-aldehyde by the shoot tips of induced and noninduced field pennycress plants was compared. Both the rate of metabolism and the qualitative pattern of metabolites produced six hours after application were similar in induced and noninduced plants. The 2 major metabolites were identified by GC-MS as GA_{12} and an isomer of GA_{19}. This latter compound, however, does not appear to be native to field pennycress. Small amounts of ^{14}C-GA_{12}-aldehyde were also incorporated into $GA_{19, 20, and\ 44}$. In addition, a radioactive compound with chromatographic properties similar to GA_9 was observed in plants from both treatments. These results coupled with our previous studies on kaurenoic acid metabolism indicate that the limiting step(s) in GA biosynthesis in noninduced field pennycress shoot tips lies between kaurenoic acid and GA_{12}-aldehyde.

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PURIFICATION OF GIBBERELLIN$_{53}$-OXIDASE FROM SPINACH

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Spinach is a long-day rosette plant, in which stem growth is mediated by gibberellins. It has been shown that two enzymatic steps, GA_{53}-oxidase and GA_{19}-oxidase, are controlled by light. To develop an understanding into this light regulation, purification of GA_{53}-oxidase has been undertaken. The original assay relied on the HPLC separation of the product and substrate, but was considered too slow for the development of a purification scheme. A TLC system was developed which in conjunction with improvements to the assay conditions was sensitive and gave rapid results. The partial purification of the GA_{53}-oxidase is achieved by a high speed centrifugation, 40-55% ammonium sulphate precipitation, an hydroxyapatite column, Sephadex G-100 column and an anion exchange FPLC column, Mono Q HR 10/10, yielding 1000-fold purification and 15% recovery. Monoclonal antibodies to the protein will be raised and used to further characterize this enzyme. Supported by the U.S. Department of Energy and a U.S.D.A. grant.

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ENDOGENOUS GIBBERELLINS AND STEM GROWTH AS RELATED TO PHOTOPERIOD IN *SILENE ARMERIA* L.

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The early 13-hydroxylation gibberellin (GA) pathway operates in the long-day plant *Silene armeria* grown under both long-day (LD) and short-day (SD) conditions. Thus, induction of stem growth must be related to quantitative changes in the GA pattern. Using GC-SIM-MS and GAs labeled with stable isotopes as internal standards, the levels of GA_{53}, GA_{19}, GA_{20}, and GA_1 were measured in shoots and various organs of plants grown under different photoperiods. Exposure to 8 LD decreased the levels of GA_{53} and GA_{19}, and increased the levels of GA_{20} and particularly of GA_1; the latter GA accumulated to very high levels in expanding leaves and tips. When plants were exposed to LD, followed by SD, GA levels decreased, and the relative increases in stem length were correlated with the level of GA_1 at the time the plants were returned to SD. These observations suggest that GA_{53}-oxidase, and probably also GA_{19}-oxidase, are under photoperiodic control. Furthermore, GA_1 appears to be active *per se* in *Silene* in causing stem growth, since its level was always correlated with the degree of stem elongation. Supported by the U.S. Department of Energy and by a U.S.D.A. grant.

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GIBBERELLINS AND HETEROSIS IN MAIZE: QUANTITATIVE RELATIONSHIPS

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Shoot growth, response to exogenous GA_3, and the endogenous concentration of GA_1 and GA_{19} were studied in a group of maize inbreds and their hybrids. Heterosis was observed in all hybrids as their shoot dry weights, heights and leaf areas exceeded those of the parental inbreds. Exogenous GA_3 promoted elongation of all genotypes but the inbreds were more responsive than the hybrids. The inbreds contained lower concentrations of endogenous GAs than the hybrids. Regression analyses indicated highly significant linear relationships between shoot growth and the log of endogenous GA concentration. A signficant negative correlation was also observed between endogenous GA concentration and sensitivity to exogenous GA_3. Collectively, these results indicate a role of endogenous GAs in the regulation of heterosis in maize. Inbreds grow slowly in part due to a deficiency of endogenous GA whereas hybrids grow faster due to increased GA content. This relationship suggests that exogenous GA_3 could improve the performance of maize inbreds which are grown to produce hybrid corn seed.

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GIBBERELLIN (GA) BIOSYNTHESIS IN ELONGATING INTERNODES OF ZEA MAYS (MAIZE): THE 3β-HYDROXYLASE

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The early-13-hydroxylation pathway for GA biosynthesis in maize leads to GA_1, the main GA responsible for shoot elongation. Growth response data, double-labeled feeding studies and endogenous GA levels suggest that the dwarf-1 mutant blocks the step GA_{20} to GA_1 (working with intact seedlings). We are screening for a system from maize from which we can purify the 3β-hydroxylase and study its physical and biological properties. We find that diced internodal (cortical) tissues will metabolize [^{13}C,^{3}H]-GA_{20} to [^{13}C,^{3}H]-GA_1 and [^{13}C,^{3}H]-GA_{29} with metabolism as high as 80%. A "cell free" system from this material will give 5% metabolism. (Identification of metabolites is by GC-SICM). In a typical experiment 1g of internodal tissue is frozen in liquid nitrogen and macerated in 0.1M Tris plus cofactors. The homogenate is centrifuged at 15000 x g for 30 min at 4°C and the supernatant used for metabolic studies.

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ELONGATING INTERNODES OF ZEA MAYS (MAIZE): EARLY STEPS IN THE GA BIOSYNTHETIC PATHWAY.

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The early steps in the gibberellin (GA) biosynthetic pathway have yet to be defined for tissues that show a growth response to GAs. To this end we have synthesized the [^{13}C,^{3}H]-ent-kaurenoids, ent-kaurenol, ent-kaurenal and ent-kaurenoic acid. We also have double-labeled ent-kaurene and double-labeled GA_{12}-aldehyde. We feed 1 - 10μg of each substrate, individually, to 1.0g diced internodes (cortex) in the appropriate buffer plus cofactors. We have observed up to 80% metabolism. We have identified (full scan GC-MS) 7β-hydroxy-ent-kaurenoic acid as the major metabolite from double-labeled ent-kaurenoic acid feeds, thus defining the step ent-kaurenoic acid to 7β-hydroxy-ent-kaurenoic acid.

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GIBBERELLINS FROM MAIZE CALLUS CULTURES

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The purpose of this study is to evaluate the role of gibberellins (GAs) in the growth and differentiation of maize callus. We are identifying the GAs found in undifferentiated (Type II) callus in addition to embryos and seedlings derived from these cultures. Wild-type (A188/B73) and mutant (*d1*; A188) embryos, 10-15 days old, are cultured on N6 medium supplemented with L-proline, casamino acids and 2,4-D. Subsequent callus is subcultured every 1-2 weeks onto fresh medium. Wild-type callus (300g) has been extracted with 80% methanol, purified by solvent partitioning and immunoaffinity chromatography. The fractions from the immunoaffinity column have been analyzed by GC-MS (SICM) and shown to contain the following gibberellins: GA_{53}, GA_{44}, GA_{19}, GA_{17}, GA_{20}, GA_{29}, GA_1, GA_5 and GA_3.

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GIBBERELLIN CONTENT OF *Na/na* GRAFTS OF *PISUM*

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The *na* allele of *Pisum* blocks GA biosynthesis in the shoots, resulting in a severely dwarfed (*nana*) phenotype. Grafting *Na* genotypes to *nana* stimulates elongation of *nana* internodes. We measured GA content by GC-SICM analysis of plant extracts containing internal standards of gibberellins A_1, A_8, A_{19} and A_{20}. Apices of intact *na le* contained 7-10% of the GA_1, about 0.5-1% of GA_{19} and GA_{20} and about 5% of GA_8 found in *Na le* (dwarf) apices. In grafts of *Na* donors to *na le* receivers, both internode length and GA_1 content of *na le* were comparable to those of *Na le*. GA_8 content was 1-3x higher in grafts than in intact *Na le*. Levels of GA_{19} and GA_{20} in *na le* were increased in grafts, but were still only 2-3% of the content in *Na le*. In *Na Le* (tall)/*Na le* and *Na le*/*Na le* grafts, GA_1 content and growth of the *Na le* receivers were unchanged. We conclude that GA_1 levels are tightly regulated by the *Le* locus. GA_{20} appears to be the major translocated gibberellin.

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GIBBERELLIN METABOLISM IN ISOLATED PEA FRUIT TISSUE AND INTACT FRUITS.

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Gibberellins (GAs) have been shown by others to be required for normal development of pea fruit. Whether the pericarp of the developing pea fruit produces GAs in situ is not known. To determine if the pericarp has the capacity to produce GAs during fruit growth, the metabolism of the first two committed GAs in the biosynthetic pathway, [^{14}C]GA_{12}-aldehyde and [^{14}C]GA_{12} was examined in tissue obtained from pollinated, parthenocarpic, and control (emasculated) fruit over 4 days from treatment. [^{14}C]GA_{12}-aldehyde was converted primarily to conjugates, including [^{14}C]GA_{12}-aldehyde conjugate. [^{14}C]GA_{12} was converted to [^{14}C]GA_{53} in all tissue, but by day 4 only tissue from pollinated or parthenocarpic fruits (which continued to elongate) showed sustained formation of [^{14}C]GA_{53}. When [^{14}C]GA_{12} is applied to 4-day-old fruits attached to the plants, the major product obtained after 24 hours is [^{14}C]GA_{20} (as identified by GC-MS). No transport to the developing seed was observed. These results indicate that the elongating fruit tissue has the capacity to produce GAs.

This work was supported in part by the Minnesota Agricultural Experiment Station and the National Science Foundation - NSF/DMB-8607749.

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PARTIAL PURIFICATION OF KAURENE OXIDASE FROM MICROSOMAL PREPARATIONS OF *Gibberella fujikuroi*.

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The oxidation of kaur-16-ene is catalyzed by a microsomal cytochrome P-450 linked mixed function oxidase. Kaurene oxidase activity and GA3 content were monitored in liquid cultures of *Gibberella fujikuroi*. In addition, active enzyme was partially purified from crude microsomal preparations.

Kaurene oxidation was evident in one-day-old suspended cultures. Activity increased at two days, then declined in subsequent days. GA3 content was very low in the filtrate of one-day-old cultures, but steadily increased with culture age. Full enzyme activity was recovered after a crude microsomal preparation was passed through a Sephadex G-25 column. Sodium cholate-treated microsomes were chromatographed on a column of Sepharose 4B linked to octyl amine. High kaurene oxidase activity was found in fractions that possessed high cytochrome c reductase activity, but only seven percent of the total protein, resulting in a substantial enrichment of specific activity.

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QUANTITATION OF INDOLE-3-ACETIC ACID IN CARROT CELL SUSPENSION CULTURES

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Carrot cells in suspension grow well on MS medium supplemented with 1 mg/l of 2,4-D. When transferred to 2,4-D-free medium, the cell clusters usually differentiate and eventually form somatic embryos. We determined that omitting 2,4-D from the medium increased production and excretion of IAA (Table). 2,4-D seems to also inhibit the formation of esterified IAA. The significance of these metabolic changes in relation to the process of embryogenesis remains to be clarified.

	Proliferating culture		Embryogenic culture	
	cells	medium	cells	medium
	ng/g	*ug/l*	*ng/g*	*ug/l*
Free IAA	**17.0**	**2.7**	**28.9**	**6.8**
Free + ester IAA	**19.4**	**8.5**	**61.0**	**12.9**
Total IAA	**410.5**	**9.4**	**615.0**	**14.7**

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EVIDENCE FOR IAA BOUND TO ZEIN

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Indole-3-acetic acid (IAA) was reported over 40 years ago to be released by alkaline hydrolysis from zein, the dominant storage protein in corn. Bioassays were used in the analyses. The present work uses western blot analysis and combined gas chromatography-mass spectrometry (GC-MS) to confirm the presence of IAA in zein. Protein extracted from protein bodies isolated from mature corn kernels and commercially available zein were subjected to SDS-PAGE and transblotted using a monoclonal antibody against carboxyl-linked IAA. Major bands corresponding to 22 and 19 kD tested positive for IAA. The laboratory-prepared and commercial zein were also base-hydrolyzed and extracted for IAA. Mass spectra and elemental composition confirmed the presence of IAA. The nature of the linkage of IAA to the protein is under investigation.

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PROPERTIES OF THE NPA BINDING SITE AND ITS LOCALISATION IN MAIZE ROOTS

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A number of reports have described the physiological effects of NPA (N-1-naphthylphthalamic acid) treatment of plants. These effects include an inhibition or delay in tropistic reactions, and the inhibition of IAA efflux and calcium influx across the plasmalemma. The results of a study of (^{3}H)NPA binding to a membrane fraction (5000g supernatant; 48000g pellet) prepared from maize roots are presented. Comparing such preparations from different parts of the root, binding activity was found to be preferentially localised in the elongating zone (ie. where differential growth occurs during gravitropism). When membranes were fractionated on sucrose density gradients, (^{3}H)NPA binding coincided with plasmalemma markers. Binding sites were solubilised from the total membrane preparation and from plasmalemma-enriched fractions using the non-ionic detergents Nonidet NP40 or Triton X-100 and solubilised activity was assayed using polyethyleneimine (PEI) treated filters. Similar results were obtained with Sephadex PD10 columns, but the PEI method is more rapid. The characteristics of (^{3}H)NPA binding to the 5000-48000g fraction and to the solubilised site are compared.

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INDOLE-3-ACETIC ACID METABOLISM DURING BEAN SEED DEVELOPMENT AND GERMINATION.

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The ratio of the level of free IAA to conjugates changes drastically at different stages of bean seed development. Rapidly growing seeds (1-2 weeks post-anthesis) are characterized by a high level of free IAA, and, in the youngest seeds, also relatively high levels of ester IAA. The decrease in free and ester IAA in later stages of seed development is accompanied by a rapid increase in the content of amide IAA--which accounts for 80% of the total IAA pool in mature seeds. Rapid accumulation of amide conjugates occurs during the period of storage protein synthesis throughout the cotyledon stage of seed development and the first stage of maturation. The amide conjugates present in mature beans were shown to be a series of immunologically related peptides of 3-25 kDa. In order to gain more knowledge about the role of these IAA peptides in bean seeds we have investigated their fate during different stages of germination, using immunological methods for their detection on Western blots and mass spectrometry for quantitation. *This work is supported by a USDA-CRGO Plant Growth and Development grant 85-CRCR-1-1718*

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INCREASED IAA TRANSPORT IN AXILLARY BUDS UPON RELEASE FROM APICAL DOMINANCE

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To investigate the transport of indoleacetic acid (IAA) simultaneously in the stem and the axillary bud, bud-bearing nodal stem segments of Phaseolus vulgaris L. were excised and agar blocks containing ^{14}C-IAA or ^{3}H-IAA were placed on the apical cut surface and the bud stump respectively. A plain receiver block was placed on the basal end. After a period of transport, the stem segment and the attached bud stump were sectioned, and the activity of sections and agar blocks was counted. We found that the transport of ^{3}H-IAA from the bud stump to the receiver was greatly accelerated in plants decapitated one or two days prior to the experiment, compared to the intact controls. Decapitation also caused a decrease in the ability of the stem axis to transport ^{14}C-IAA from the apical to the basal end of the stem segment. The increased ability of the axillary bud to transport IAA, relative to that of the stem axis, may play a role in the release of the bud from apical dominance.

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IDENTIFICATION OF THE CELLS INVOLVED IN AUXIN TRANSPORT IN MAIZE MESOCOTYL

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A study was undertaken to identify by a direct method the cells involved in auxin transport through maize mesocotyl tissue. The auxin photoaffinity labeling agent, 7-[^{3}H],5-azidoindole-3-acetic acid (N_3IAA), was loaded into excised stem tissue from a cut end. Polar transport of this analog was demonstrated over 4 hours by comparing uptake into tissue loaded with N_3IAA from the apical vs. the basal end. Triiodobenzoic acid, an auxin transport inhibitor, inhibited N_3IAA uptake into tissue. Tissue which had taken up the photoaffinity labeling agent was photolyzed to covalently fix the radioisotope within cells. This tissue was sectioned and subjected to in situ autoradiography. The outermost cell of epidermal tissue and certain files of cells in vascular tissue were densely labeled indicating that on a per cell basis these two cell types are most actively transporting auxin.

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CAUSES OF AUXIN-INDUCED ELECTRIC SURFACE POTENTIALS IN COLEOPTILE AND OTHER GROWING SHOOT SEGMENTS

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Among the earliest reported auxin effects was the development of a positive surface potential (SP). Time course studies show that the SP changes at the same time as there is (a) an acidification of the apoplast and (b) a re- or hyperpolarization in the transmembrane potential (TMP) of the epidermal cells. Different treatments were used to assess, whether SP changes are generated only in the cell walls or also by TMP differences via the symplast. The changes induced in the electrical resistance and in the magnitude of SP generation allow determination of the symplastic share of overall SP generation. The results support a contribution of symplastic TMP differences, being within the same range as SP differences generated in the apoplast.

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EVIDENCE FOR REGULATION OF POLAR AUXIN TRANSPORT AT THE EFFLUX CARRIER IN MAIZE COLEOPTILE SECTIONS

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Previously we have shown that conditions which result in an increased auxin-induced growth response in maize (Zea mays, L.) coleoptile sections also result in a decrease in the velocity of polar auxin transport. Coleoptile sections (2 mm) given conditions (2 h pre-incubation) which result in slower transport of IAA have different kinetics for net IAA accumulation compared to sections given conditions (0.5 h pre-incubation) which result in faster transport. In further experiments, sections were loaded with 30 nM (^{3}H)IAA in the presence of increasing unlabeled IAA at low pH. Efflux of (^{3}H)IAA was then followed as a function of unlabeled IAA. Saturation of efflux appears to occur at a lower conc. of IAA in sections showing slower transport.

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EFFECT OF CALCIUM AND AUXIN ON ETHYLENE INDUCED RADIAL EXPANSION IN PEA EPICOTYLS

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Ethylene application to four-day-old seedlings of etiolated pea [*Pisum sativum* L. var. Alaska] induces a rapid decrease in elongation growth and increases in radial expansion of subapical internode tissue. Transducer and optically enhanced video imaging measurements indicate that elongation growth slows within six to ten minutes and radial expansion begins within fifteen to twenty minutes after optimal ethylene exposure [1-3 µl/l]. Growth redirected by ethylene, from elongation to the radial dimension demonstrates insensitivity to acid [pH 4-5] but retains ability to respond to auxin. Measurements, based upon weight to length ratios, of seedlings given ethylene in combination with auxin show greatly increased radial growth capability. Seedlings grown in high calcium concentations, show slower elongation growth, but enhanced ability for ethylene induced swelling. Radial growth induced by ethylene may proceed by mechanisms which differ from elongation growth.

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RELATIONSHIP BETWEEN CONCENTRATION OF FREE IAA IN COLEOPTILE SECTIONS AND THE MAGNITUDE OF THEIR GROWTH RESPONSE

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The results of our experiments indicate that the growth of 5 day old Zea mays L. coleoptile sections is not necessarily related to the amount of internal free IAA. [^{3}H]IAA (concentration from 0.1 uM to 100 uM) was applied, in the presence and absence of NPA, to 1-cm coleoptile sections showing low responsiveness and high responsiveness to applied IAA. Following 120 min incubation, total growth of the sections and their accumulation of radiolabel were measured. This accumulation was corrected to express only free IAA. In general, all treatments showed that growth and accumulation increased with increasing concentration of applied IAA. However, when growth was expressed in terms of IAA accumulated, it was found to decrease as accumulation of applied IAA increased.

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OXIDATION OF INDOLE-3-ACETIC ACID TO OXINDOLE-3-ACETIC ACID BY ETIOLATED AND GREEN CORN TISSUES

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Etiolated corn tissues oxidize indole-3-acetic acid (IAA) to oxindole-3-acetic acid (OxIAA) (Plant Phys. 71, 211). This oxidation results in loss of auxin activity and may play a role in regulating IAA-stimulated growth. The enzyme has been partially purified and characterized and shown to require O_2, and a heat-stable lipid-soluble corn factor which can be replaced by linolenic or linoleic acids in the oxidation of IAA. Corn oil was tested as a cofactor in the IAA oxidation reaction. Corn oil stimulated enzyme activity by 30% while trilinolein was inactive. The capacity of green tissue to oxidize IAA was examined by incubating leaf sections from 2 week old light-grown corn seedlings with ^{14}C-IAA. OxIAA and IAA were separated from other IAA metabolites on a 3 ml anion exchange column. Of the IAA taken up by the sections, 13% was oxidized to OxIAA. This is the first evidence that green tissue of corn may also regulate IAA levels by oxidizing IAA to OxIAA. (Supported by NASA Researchers Associate Award)

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HORMONAL RELATIONS OF RADIATION-INDUCED TUMORS OF *ARABIDOPSIS THALIANA*

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When gamma-irradiated *Arabidopsis* seed was germinated, tumors appeared on hypocotyls and apical meristems of the resulting plants. Several tumors have been cultured on hormone free medium for over two years since excision from the plants. The tumor lines display a range of phenotypes (shooty, rooty, undifferentiated) suggestive of abnormal hormone balance. To determine whether hormone overproduction or hypersensitivity is involved in tumorigenesis, we are measuring hormone levels in the tumor lines and characterizing their response to exogenously supplied growth regulators. Growth of two tumor lines is stimulated by either NAA or BAP (at concentrations optimal for growth of normal *Arabidopsis* callus tissue), one is stimulated by NAA only, two by BAP only, and one is stimulated by neither. Growth of all lines tested thus far is inhibited by gibberellic acid, ethephon and ACC. The tumor lines appear more sensitive to ACC than normal callus tissue. Most tumors studied to date appear unlikely to have arisen due to increased hormone sensitivity. Experiments are in progress to determine auxin and cytokinin levels in the tumor lines.

Supported by DOE grant DE-FG02-88ER13907

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HYDROLYSIS OF INDOLE-3-ACETIC ACID ESTERS EXPOSED TO MILD ALKALINE CONDITIONS.

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Indole-3-acetic acid is present in plant tissues as the free acid and also in the form of covalently linked conjugates,i.e., ester and amide. Ester conjugates (including ester, acyl anhydride, and S-acyl anhydrides) are hydrolyzed easily in basic solutions, however, few studies have analyzed the relationship between pH and rate of hydrolysis of the known ester conjugates. Use of basic conditions during extraction or purification by several laboratories using various protocols suggested that a more systematic analysis of this process was needed. In this report we present data indicating: 1) hydrolysis of IAA-glucose of greater than 1% between 2 to 4 h at pH 9 and 9.3, and, greater than 2% at pH 9.5; 2) lability of some ester conjugates is even greater than that of IAA-glucose under mild alkaline conditions; and 3) hydrolysis of IAA-glucose (1%) and IAA-*p*-nitrophenol (58%) in the three phase extraction system described by Lui and Tillberg (*Physiol. Plant.* 57:441-447, 1983). These data indicate that problems with inadvertent hydrolysis of ester conjugates of IAA are apparent even at moderate pH values. *Supported, in part, by NSF Metabolic Biology grant DMB-86-17171.*

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DIFFERENTIATION BETWEEN ENDOGENOUS (^{12}C) AND APPLIED (^{14}C or ^{13}C) IAA METABOLISM DURING MAIZE ROOT GROWTH

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Intact primary maize (LG11) roots (15 ± 2 mm) were immersed (1h) in a buffered (pH 6.0) solution containing IAA (10^{-6}M, 10^{-7}M). Growth was measured 4h after treatment and three root zones (cap+apex, elongating, differentiating) were collected to determine the patterns of IAA metabolism (HPLC technique) and simultaneously level of both endogenous and applied IAA (GC-MS technique). By using HPLC separation (reversed-phase, C18) and 2-^{14}C-IAA, different patterns of metabolism were identified for each root zone. The amount of labelled IAA (always less than 50% after 4h growth) depended upon the zone considered (highest in differentiating zone) and the concentration used. Radioactivity was mainly distributed into two peaks: IAA and an unidentified polar substance (not IAA-myoinositol). GC-MS technique was used to quantify level (free and ester-bound) of both endogenous ^{12}C-IAA and applied ^{13}C-IAA after immersion and 4h of growth. From data obtained for treated and untreated roots, the role of IAA metabolism during root growth is discussed.

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STRUCTURAL ANALYSIS OF AMINO ACID CONJUGATES OF INDOLE-3-ACETIC ACID

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Conjugates of indole-3-acetic acid (IAA) participate in auxin homoeostasis, transport and storage. The molecular mechanisms involved recognize these compounds by their threedimensional structures. We have therefore examined the naturally occuring N(indole-3-acetyl)-aspartic acid and some other, synthetic, IAA amino acid conjugates by crystallographic and NMR methods. The data indicate (preliminarily) that the hydrophobic tails of the long-chain aliphatic amino acids tend to cluster around the indole ring while -CONH- and -COOH groups converge into a hydrophilic pole, rendering the molecules increasingly amphipatic. This appears to correlate with a decrease in physiological activity in tissue culture, where the conjugates are hydrolyzed to act as sources of auxin.

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CHARACTERIZATION OF A 22-KILODALTON SUBUNIT AUXIN-BINDING PROTEIN FROM MAIZE

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A 22-kDa subunit auxin-binding protein (ABP) in maize is shown to share properties of the auxin-binding activity in crude maize membrane extracts designated Site I. Polyclonal antibodies prepared against this protein were used in immunoblot analysis to determine the subcellular and tissue location of ABP in maize shoots and to quantify the relative abundance of ABP in maize seedlings. Like Site I, the ABP comigrates in isopycnic centrifugation with the endoplasmic reticulum marker, cytochrome c reductase. The ABP is most abundant in the growing region of the mesocotyl. Furthermore, phytochrome-mediated changes in Site I binding correlate with the relative changes in the abundance of the ABP. These new observations are additional support indicating that this ABP is Site I.

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CONFORMATIONAL ALTERATION OF PLANT PLASMA MEMBRANES BY THE SYNTHETIC AUXIN, 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

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Infrared spectroscopy of purified plasma membrane vesicles from hypocotyls of etiolated seedlings of soybean (Glycine max L.) provides evidence for altered protein and phospholipid conformations upon exposure to growth promoting concentrations of 2,4-D (Biochem. Biophys. Res. Commun. 147, 506-512, 1987). The changes include a concentration dependent broadening of amide I absorbance and a change in the absorbance ratios of amide I and amide II indicative of a change in protein conformation at 2,4-D concentrations as low as 10 nM. An alteration in the vibrational frequency of the choline stretch was seen over the range 1 μM to 1 mM but an alteration in hydrocarbon chains (CH_2 scissoring) was seen only at 1 mM 2,4-D. Additional evidence for a conformational change in the plasma membrane in response to auxin is provided from labeling studies with impermeant membrane-reactive reagents.

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LOCALIZATION OF IAA-ASPARTATE TO PLANT CELL VACUOLES

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The intracellular localization of IAA-asp, produced from exogenous IAA, was studied in red beet root tissue. IAA-asp synthesis was induced by incubating strips of beet root tissue in 100 mM phosphate buffer (pH 6.0) containing 300 uM IAA. Vacuoles rapidly isolated from the IAA-treated tissues were lacking cytoplasmic enzymes but contained IAA-asp. The ratio of IAA-asp to the vacuolar pigment, betanin, was the same in the isolated vacuoles and in the IAA-treated tissues. Furthermore, the efflux of IAA-asp from IAA-treated beet tissue was followed after exposure of the tissue to increasing concentrations of DMSO to increase membrane permeability. The efflux of IAA-asp and the vacuolar pigment, betanin, paralleled each other indicating that both compounds were localized in the same compartment. Thus, in red beet root tissue, IAA-asp synthesized from exogenous IAA appears to be localized in the cell vacuole.

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AUXIN PHYSIOLOGY OF THE TOMATO MUTANT DIAGEOTROPICA

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The tomato (Lycopersicon esculentum, Mill.) mutant, diageotropica (dgt), exhibits biochemical, physiological, and morphological abnormalities which suggest these plants may have a defect associated with a primary site of auxin perception or action. We have investigated aspects of the auxin physiology and growth parameters of dgt and wild-type (VFN8) seedlings. The rate of basipetal indole-3-acetic acid (IAA) polar transport is identical in the two varieties, but dgt sections have a slightly greater capacity for IAA transport. 2,3,5-triiodobenzoic acid (TIBA) and ethylene reduce transport in mutant and wil-type sections. The kinetics of auxin uptake into VFN8 and dgt sections are nearly identical. These results make it unlikely that an altered IAA efflux carrier or IAA uptake symport are responsible for the pleiotropic effects resulting from the dgt mutation. Measurements reveal that the osmotic potential of dgt cells is more negative than fluid expressed from VFN8. Thus turgor in dgt cells is adequate to drive cell extension suggesting this growth parameter is not responsible for the inability of dgt sections to elongate in response to IAA. Auxin treatment causes an increase in plastic wall extensibility (i.e. wall loosening) in VFN8 sections but has no effect on dgt sections. These data imply dgt hypocotyls suffer a defect which prevents them from initiating a key event which culminates in auxin-induced cell wall loosening.

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THE *DIAGEOTROPICA* MUTANT OF TOMATO LACKS HIGH SPECIFIC ACTIVITY AUXIN BINDING SITES

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Tomato (*Lycopersicum esculentum*, Mill) plants homozygous for the single gene *diageotropica* (*dgt*) mutation have reduced shoot growth, abnormal vascular tissue, altered leaf morphology, and lack of lateral root branching. These and other morphological and physiological abnormalities suggest that *dgt* plants are unable to respond to the plant growth hormone auxin (indole-3-acetic acid, IAA). The photoaffinity auxin analogue ^{3}H-5N_3-IAA specifically labels a polypeptide doublet of 40 and 42 kD in membrane preparations from stems of the parental variety VFN8, but not from stems of *dgt*. In elongation tests, excised *dgt* roots respond in the same manner to IAA as VFN8 roots. These data suggest that the two polypeptides are part of a physiologically important auxin receptor system which is altered in a tissue-specific manner in the mutant.

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INBRED LINES OF *LEMNA GIBBA* SUITABLE FOR USE FOR MUTANT SELECTION IN PHYTOHORMONE RESEARCH.

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Our Laboratory has isolated several mutants from the parent line of *Lemna gibba* G-3 obtained from Dr. C.F. Cleland (see *Plant Physiol* 86:522-526). One of these has a chl b deficient phenotype, another is an IAA overproducer. Classical genetic studies with these mutants is made more difficult by the fact that they were not obtained in inbred lines. Since no such lines were available, we began a breeding program to produce lines suitable for mutagenesis and selection for biochemical mutations. We now have several such lines. During this process we obtained lines with what appear to be changed requirements for daylength regulation of flowering. We are now concentrating our selection schemes on hormone metabolism abnormalities. *Supported by NSF Metabolic Biology grant DMB-86-17171.*

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INDOLE-3-ACETIC ACID AND THE SLENDER PEA PHENOTYPE

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The slender phenotype of pea exhibits rapid internode elongation and other morphological characteristics which resemble a pea treated with a saturating GA dose. While the gene combination la cry^S determines the slender phenotype, the genes le and na decrease syntheses of GA_1, and all GAs, respectively. Three genotypes of slender pea were studied: Line 188 (le la cry^S na), Line 197 (le la cry^S Na), and a third slender selection (le la cry^S Na). While treatment with GA biosynthesis inhibitors did not inhibit growth, antiauxins greatly reduced internode elongation. Since GA_1 content is not related to slender stem growth, auxin levels in the apical buds and stem elongation zones were measured by GC-MS with $^{13}C_6$-IAA as an internal standard, and were compared with those of non-slender genotypes.

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MUTANTS OF *ARABIDOPSIS* RESISTANT TO ALPHA-METHYL TRYPTOPHAN

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One of our approaches to studying auxin biosynthesis is to isolate mutants with altered metabolism of tryptophan, the probable precursor of indole-3-acetic acid (IAA). In both microorganisms and plant tissue culture, mutants resistant to 5-methyl tryptophan (5-MT) frequently have elevated levels of tryptophan and are sometimes auxin autotrophic in culture. Nine *Arabidopsis* mutants showing varying levels of resistance to α-methyl tryptophan (αMT) were isolated by screening approximately 10,000 mutagenized seed (M2) on αMT at 100 to 300 μM. One of the more resistant mutants (amt-1) is being characterized in greater detail. The mutant plants are morphologically normal and are able to germinate and grow on agar containing 25 μM αMT, a concentration which completely inhibits the growth of wild-type. Cultured mutant callus is resistant to concentrations of 100 μM αMT or more. The mutant shows cross-resistance to 5-MT but is sensitive to 5-methyl anthranilic acid. Preliminary data suggest that the mutation in amt-1 is dominant. Experiments are in progress to map the mutant and to characterize the defect at the physiological and biochemical levels. We hope to clone the mutant allele by transforming tobacco callus to αMT resistance using a cosmid library prepared from the mutant DNA.

Supported by USDA grant 87-CR-CR-1-2559

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RESPONSES OF ARABIDOPSIS TO IAA-AMINO ACID CONJUGATES

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Responses of wild-type Arabidopsis thaliana var. Columbia to IAA and various IAA-amino acids were tested for comparison to IAA conjugation mutants we have selected. Although germination was not affected, seedling root growth was inhibited by free IAA more than by any of the conjugates tested. IAA-asp and IAA-asn had no detectable effect on root growth whereas IAA-ala, IAA-phe, IAA-gly, and IAA-leu had intermediate effects. Plants grown up to 5 weeks in the presence of free IAA concentrations of 100 uM or greater showed abnormal shoot development and failed to mature. In the presence of IAA-phe, IAA-gly, and IAA-leu at 300 uM or greater plants were similarly affected until after about two weeks when the tissues began to callus. In contrast, 500 uM or greater IAA-asp had almost no effect on plant growth, although minor epinasty of the leaves was occasionally observed. Uptake studies indicate that differential uptake does not account for the variation in the activities observed.

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A STEREOCHEMICAL MODEL FOR CYTOKININ ACTIVITY

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The three-dimensional structures of several cytokinins were compared and a stereochemical model for cytokinin activity is proposed. Three-dimensional X-ray crystallographic coordinates of eight N^6-substituted adenines and adenosines were initially examined graphically using a computer and subsequently, values for the torsional angles of the side chains of the molecules were calculated. Cytokinin bases, f^6Ade, i^6Ade and b^6Ade, are all observed to crystallize isomorphously and have extremely similar three-dimensional conformations. Although some cytokinins such as 2MeS i^6Ade and b^6Ado do not exhibit the preferred orientation about ϕ_1, model-building studies suggest that these molecules have unrestricted freedom of rotation about ϕ_1 and can achieve the conformation observed for the N^6-substituted adenines. Very weak cytokinin activity for *cis*-zeatin may be explained by the existence of a strong hydrogen bond between the OH group of *cis*-zeatin and the N1 of the adenine, while it is conformationally impossible to form a similar hydrogen bond for the active cytokinin, trans-zeatin. N1 of the adenine ring is known to be essential for activity.

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IMPACT OF CYTOKININ LEVELS IN XYLEM SAP ON LEAF LEVELS

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Several authors have suggested that the level of cytokinins transported from roots to shoots in xylem sap decreases when roots are stressed and that a resultant decline in leaf cytokinin affects leaf function. When the roots of bean plants are deprived of oxygen, stomata close and leaf growth rates are reduced. An objective of this study was to determine the influence of root hypoxia on the levels of three cytokinins (ZR, DHZR, 9RIP) in xylem sap and the impact of sap changes on leaf cytokinin levels. Roots of intact plants were either aerated or bubbled with nitrogen gas in solution culture for 3 or 24 hours. The levels of ZR, DHZR and 9RIP in leaves and xylem sap expressed from the root system were determined. The levels of all three cytokinins in the xylem sap declined within 3 hours of the stress. In leaves, endogenous levels of ZR did not differ appreciably (aerated 259 pm/g; hypoxic 243 pm/g) between aeration treatments, while levels of 9RIP and DHZR increased in response to the stress.

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CYTOKININ ENHANCED ACC UTILIZATION DURING RELIEF OF THERMOINHIBITION AND OSMOTIC RESTRAINT IN LETTUCE SEEDS

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The thermoinhibition (TI) at 30°-35°C of pregermination C_2H_4 production and germination in Mesa 659 lettuce seeds was synergistically alleviated by 0.05 mM kinetin (KIN) and 10 mM 1-aminocyclopropane-1-carboxylic acid (ACC). At 15°-20°C, KIN+ACC showed no synergistic effect. At 35°C, 0.05 mM KIN generated a greater germination potential (germinated in lower osmotic potential PEG-8000 solutions) than 10 mM ethephon (ETH) and together with ACC or ETH synergistically alleviated TI. ETH was more active than KIN or KIN+ACC in removing osmotic restraint (OR) (by -0.06 MPa PEG solution) at 25°C, in spite of the additive effect of KIN+ACC. The ACC uptake by the seed was not influenced by KIN. Upon slitting the seed at the cotyledon end following the uptake of chemicals, ACC was readily converted to C_2H_4 and the synergistic or additive effects of KIN+ACC were lost. Thus, the seed coat integrity and the KIN enhanced ACC utilization may be the bases for the synergistic or additive effects of KIN+ACC in alleviating TI and OR.

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CYTOKININ LEVELS IN MAIZE XYLEM EXUDATE AND DEVELOPING KERNELS

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Cytokinins may influence starch grain and endosperm cell number and thus affect final kernel size. Our objectives were 1)to determine cytokinin levels during maize kernel development, and 2)to compare cytokinin levels in xylem exudate and kernels. Cytokinins were purified by immunoaffinity chromatography, and quantified using HPLC-DAD. Cytokinin levels, particularly Z and ZR, increased dramatically, peaking at 8-10 days after pollination, and rapidly decreased. Cytokinin levels in xylem exudates could not account for the rapid increase in kernel cytokinin levels. Also, cob tissue cytokinin levels were lower than would be expected if the sudden increase in kernel cytokinins was due to increased root production and transport. Two explanations are 1)very high levels of cytokinin glucosides not recognized by the antibodies are being transported in the xylem exudate, or 2)kernels are synthesizing cytokinins. We are currently testing these hypotheses.

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PURIFICATION AND CHARACTERIZATION OF THE ETHYLENE BIOSYNTHESIS-INDUCING XYLANASE FROM TRICHODERMA VIRIDE

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The ethylene biosynthesis-inducing endoxylanase (EIX) synthesized by Trichoderma viride in response to growth on xylan has been purified to homogeneity in high yield by ultrafiltration and ion-exchange chromatography. Physical characterization of this glycoprotein has been performed by amino acid analysis, GC/MS analysis of the attached glycosyl groups, and peptide mapping. Products released from commercial xylan and crude cell wall preparations after treatment with EIX were reduced with $NaBD_4$ and analyzed by thin-layer chromatography and GC/MS to determine cleavage sites and products. Kinetic constants, as well as temperature and pH parameters, have been determined.

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UNICONAZOLE REDUCES BOTH ENDOGENOUS AND TRICLOPYR-INDUCED ETHYLENE IN BEAN SEEDLINGS

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Uniconazole applied as a soil drench to mung beans (Phaseolus aureus) at the time of planting or to 17-day-old bean (Phaseolus vulgaris) seedlings, significantly reduced ethylene levels in both species. Control plants treated with triclopyr (0.8 g/ha), an auxin-like herbicide, showed typical symptoms of auxin injury. This included leaf wilting, curling, epinasty and swelling of the hypocotyls associated with a dramatic rise in ethylene which reached a peak 6 h after treatment. All these symptoms of injury and the rise in ethylene induced by triclopyr were significantly decreased in the uniconazole pretreated seedlings. The present finding confirms our previous observation that the triazoles including uniconazole act as plant multi-protectants. Furthermore it indicates that the triazole-induced protection may be mediated by reducing the production of ethylene which is symptomatic of many stresses.

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ROLE OF THE 5'METHYLTHIOADENOSINE TO S-ADENOSYLMETHIONINE SALVAGE PATHWAY IN WOUND ETHYLENE FORMATION

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The mechanism by which wound ethylene is produced in (Lycopersicon esculentum Mill.) and (Cucumis sativus Mill.) fruit was studied by measuring the relative changes in 5'-methylthioadenosine (MTA) nucleosidase and 5-methylthioribose (MTR) kinase activities in relation to wound ethylene formation. MTA nucleosidase and MTR kinase activities were parallel to that of wound ethylene in both tomato and cucumber. Infiltration of tomato tissue with 5'-ethylthioadenosine, 5-isobutylthioribose, and aminoethoxyvinylglycine inhibited wound ethylene formation and MTA nucleosidase and MTR kinase activities, suggesting that the recycling of MTA to MTR and into S-adenosylmethionine is necessary for wound ethylene formation. However, infiltration of tomato tissue with IAA and polyamines had no significant effect on wound ethylene formation. These data indicate that wound ethylene is formed through the same pathway as natural ethylene, but its synthesis is not regulated by the same compounds.

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SOYBEAN ABA DISTRIBUTION AND WATER & ION UPTAKE ± STRESS

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Soybean (Glycine max [L.] Merr. cvs Ransom and Bragg) were grown in nutrient solution using a semi-automatic system to monitor water and ion uptake patterns. Finally, half of the plants were stressed with PEG 8000 (-0.3 MPa) to study the effect of stress on those patterns and ABA formation.

ABA levels in the unstressed old and young leaves and roots were approximately equal (100-200 ng g^{-1} dry wt.). Stress increased the ABA in old leaves more than young leaves (950-1000 vs 450-500 ng g^{-1} dry wt.). Stressed roots showed little increase in ABA (from 180 only to 200-240 ng g^{-1} dry wt.).

In darkness, water uptake increased during the last hours while ion uptake continued to decrease throughout the night. Breaking the darkness rapidly increased water uptake rate seven times while the increase in ion uptake was delayed, slow, and became only 3.5 times greater than in darkness. Further increase in light level raised water uptake more than ion uptake. Stress reduced the ion uptake in darkness and light (25%, 47%) less than water uptake (49%, 58%) in both cultivars.

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ATYPICAL ABSCISSION BEHAVIOR IN COTTON: A PHYSIOLOGICAL STUDY

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In cotton, the youngest still-expanding leaves exhibit the greatest sensitivity to defoliants and to the natural abscission-promoter ethylene. The physiological bases for this differential sensitivity between young and older (fully-expanded) leaves were examined. The inhibition of IAA transport through petioles by various concentrations of ethylene was equivalent at both leaf positions. Treatment with inhibitors of IAA transport and ethylene increased the speed and extent of abscission at both leaf positions but did not eliminate the differential sensitivity. Free IAA levels (per g f wt) were slightly higher in the younger leaves. Free IAA levels were reduced by ethylene treatment to a greater extent in younger leaves. Conjugated IAA levels were initially 30-100% higher than free IAA levels and were less affected by ethylene treatment. Isolated abscission-zone explants also exhibited this differential sensitivity towards ethylene.

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ABA-ALDEHYDE AND ABA-TRANS-DIOL IN APPLE FRUITS

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We have isolated ABA-aldehyde and ABA-t-diol from postharvest apple fruits, cv. 'Granny Smith' and confirmed their structure by GC-MS. These putative ABA biosynthetic precursors incorporate ^{18}O to a similar degree as ABA during 48 hours under $^{18}O_2$ atmospheres. The presence of significant amounts of ABA-aldehyde can explain the unique ^{18}O labeling pattern of ABA in this tissue, where a majority of ABA molecules containing ^{18}O is labeled in the 1'-hydroxyl group and not in the side chain carboxyl group, the primary site of incorporation for stressed leaves. Exchange of the carbonyl oxygen of ABA-aldehyde with water would decrease ^{18}O enrichment in the side chain. Results of $^{18}O_2$ experiments and feeding studies using hexadeutero-ABA-aldehyde will be presented and the biosynthetic relationship of these compounds discussed. Supported by the U.S. Department of Energy and by a U.S.D.A. grant, and by a Monsanto Graduate Fellowship to C.D.R.

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MODULATION OF THE NITRATE REDUCTASE TRANSCRIPT BY CYTOKININ AND ABSCISIC ACID IN ETIOLATED BARLEY SEEDLINGS

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To investigate the molecular mechanism of the hormonal modulation of nitrate reductase (NR) activity, the influence of benzyladenine (BA) and/or abscisic acid (ABA) on the level of NR poly(A)RNA was studied in etiolated barley seedlings using a ^{32}P-labelled NR cDNA as a probe. Enhancement of NR activity by $2x10^{-5}M$ BA was measurable only after 60 minutes of exposure of the seedlings to light, while a significant stimulatory effect on the transcript level could be clearly detected within 15 minutes. Northern blot analyses of the levels of NR poly(A)RNA indicate that the amount present is proportional to the concentration of BA ($2x10^{-8}$ to $2x10^{-4}M$) applied to the seedlings. The stimulatory effects seen for BA were nullified by ABA. The counteractive effects of ABA on BA were dose-responsive, with greater inhibition at higher concentrations of ABA. Evidence suggests that the interaction of BA and ABA on NR activity is at the transcriptional level, however, it is also possible that interactions occur at the posttranscriptional level as well.

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ON THE RELATIONSHIP BETWEEN ABA CONTENT AND ROOT GROWTH RATE

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Quantification of abscisic acid (ABA) was performed by GC-MS in elongating roots of *Zea Mays*, *Glycine max*. and *Pisum sativum*. When roots were sorted into several growth classes, a negative correlation was found between the growth rate of the root and the ABA content in its elongation zone. In a parallel experiment, the activities of different enzymes (used as markers) were tested in maize roots of selected growth classes. The activities of these markers were not related to the growth rate of the roots. Possible correlations between ABA and elongation are discussed.

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ABA AND PROLINE ACCUMULATION IN SALT-SHOCKED, SALT-ADAPTED, AND DROUGHT STRESSED ARABIDOPSIS THALIANA

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It is unknown if endogenous ABA induces free proline accumulation in plants during salt or drought stress. Endogenous ABA and free proline were measured in seedlings of Arabidopsis thaliana exposed to salt-shock, salt-adaptation, or drought stress. After salt-shock at 25, 50, and 100 mM NaCl, ABA and proline levels doubled. Above 100 mM NaCl, ABA increased linearly and proline increased by 3-fold. In salt-adapted tissue, ABA levels were highest with 75 mM NaCl and declined at higher salt concentrations. By contrast, proline accumulation increased with increased NaCl. During drought stress, ABA levels increased 9-fold within 2 hrs and reached a maximum of 15-fold by 12 hrs. Proline increased after 4 hrs and this accumulation continued over time. Initial studies have indicated that endogenous ABA induction is correlated with proline accumulation in Arabidopsis during these three different types of stress.

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IMMUNOASSAY OF BRASSINOSTEROIDS

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The principle objective of this work was to develop a specific and sensitive immunoassay for the detection and quantification of brassinosteroids (BR). A synthetic analogue of brassinolide was conjugated to a protein carrier by making a BR-succinic acid derivative and conjugating it to goat serum albumin (GSA) via the mixed anhydride method. Five BALB/c mice were injected with BR-GSA emulsified in Freund's complete adjuvant. After 2 booster injections with BR-GSA emulsified in Freund's incomplete adjuvant, a serum sample was taken from each mouse. The polyclonal antibodies activity were assayed in an enzyme linked immunosorbent assay (ELISA) using BR conjugated to mouse albumin. A competitive ELISA (CELIA) showed the polyclonal antibodies could detect and quantify free BR in the 1-100 ng range at a dilution of 1:8000. Mice with the best antibody activity were given a final aqueous boost with BR-GSA. Three days later spleen cells from these mice were fused with NS1 myeloma cells to produce hybridomas. Hybridomas were tested for activity using an ELISA and 25 positive clones were found. Characterization of the clones will also be discussed.

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BRASSINOSTEROID INDUCED EPINASTY IN TOMATO PLANTS - UPTAKE AND METABOLISM

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Previous research in our laboratory showed that brassinosteroids (BR) applied to the roots of hydroponically grown tomato plants results in an accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC) in the vegetative tissue and epinasty. In this work, we further studied BR-induced epinasty using ^{3}H-BR. Ten-day-old tomato plants were incubated in 15 ml Hoagland's solution plus 1 uM BR and 10^{6} cpm ^{3}H-BR. After incubation, the plants were rinsed and extracted with methanol. After 24 hr of incubation, there was an accumulation of 600 pM/gfw of ACC and 95,000 cpm/gfw. A time course study over 24 hr showed that as the amount of extractable radioactivity increased, there was an increase in ACC as well. Thin layer chromatography of the plant extracts on silica gel showed that when the plants were incubated up to 12 hr, radioactivity migrated as a single spot with the same Rf as the BR standard. Incubation times of greater than 12 hr resulted in radioactivity migrating to 3 spots suggesting that BR was metabolized to at least 2 products. Experiments to determine what role these metabolites play in BR-induced epinasty will be discussed.

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IDENTIFICATION OF THE BRASSINOSTEROID CASTASTERONE FROM THE CAMBIAL REGION OF SCOTS PINE (PINUS SILVESTRIS) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS), AFTER DETECTION USING A MODIFIED RICE-INCLINATION BIOASSAY.

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A modified rice-lamina inclination bioassay for brassinosteroids (BRs) synergized by use of indole-3-acetic acid to improve sensitivity(i.e. sensitive to 0.1ng brassinolide/plant) was used to detect active fractions during the purification of BRs from scrapings obtained from the cambial region of Scots pine during the period of tracheid production. After solvent partitioning and column chromatography (SiO_2, PrepPak C_{18}) and HPLC (NovaPak C_{18}), the biologically-active fractions were analyzed by GC-MS as the bismethaneboronate derivative. Castasterone was unequivocally identified by Rt on capillary GC and by mass spectrum [*m/z* 512 (M^+, rel.int. 58), 358 (29), 328 (19), 287 (20), 155 (100)]. This is the first identification of a BR from the cambial region tissues of a conifer.

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MANIPULATIONS OF THE POLYAMINE SYSTEM IN *IPOMOEA NIL* FILAMENTS.

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It has been shown that ethylene production limits filament growth in *Ipomoea nil*. Furthermore, gibberellins are known to inhibit ethylene production in filaments and thereby stimulate filament growth. Since the ethylene and polyamine biosynthesis pathways have common precursors, the present study was designed to test whether manipulation of the polyamine system could interfere with filament growth. Filaments were harvested 72 hours before anthesis and placed in vitro in the presence of potential growth regulating substances (0.1 to 100 μM). Filament growth at 30 C was monitored by using an optical micrometer. Neither putrescine nor agmatine inhibited filament growth by reducing competition for s-adenosyl-methionine (SAM). Inhibitors of polyamine synthesis (DFMO and DFMA) did not stimulate growth by increasing competition for SAM. Even when used simultaneously, these inhibitors had no significant effect. Increasing the supply of l-methionine and SAM did not inhibit growth by enhancing ethylene production. It appears that the polyamine system does not signficantly influence filament growth in *Ipomoea nil*. Further study is needed to more directly assess the influence of polyamines on ethylene production, possible compensatory changes of ethylene biosynthesis in response to these manipulations, and whether SAM availability is ever rate-limiting to filament growth.

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CONTROL OF GROWTH AND MORPHOGENESIS IN ADIANTUM PROTONEMATA BY POLYAMINES AND INHIBITORS OF THEIR BIOSYNTHESIS

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Dormant Adiantum spores on 1/10 Murashige-Skoog medium germinate after exposure to red light. The resulting cylindrical, unbranched protonemal tube elongates without cell division until the nucleus is irradiated with blue or near UV light, after which mitosis occurs. Inclusion in the medium of 0.5-1.0 mM ℓ-difluoromethylarginine (DFMA), a suicide inhibitor of arginine decarboxylase, causes a 2.5-fold acceleration of the elongation rate and the development of bumps on the protonema in red light, and a delay in cell division upon transfer to blue light. When putrescine (0.5-5.0 mM) or spermidine (0.5-1.0mM) is added to the DFMA-containing medium, the red-grown protonemal tube branches, without nuclear division or wall formation. Upon transfer to blue or white light, the nucleated branch tip stops elongating, swells and undergoes mitosis and wall formation, while the anucleate branch only swells slightly at the apex and continues elongating. The added polyamines do not produce these effects in the absence of DFMA.

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THE HORMONE-STIMULATED NADH-OXIDASE OF ISOLATED SOYBEAN PLASMA MEMBRANE IS NOT A PEROXIDASE

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The hormone-sensitive NADH oxidase, partially purified from soybean hypocotyls by Brightman et al. [Plant Physiol. 86, 1264-1269 (1988)] is not a peroxidase on the basis of recently obtained data. Plasma membranes isolated from soybean hypocotyls by two-phase partition of the microsomal fraction as described above were the starting material for spectrophotometric assays of plasma membrane NADH oxidation and peroxidase activity. Right-side out membrane vesicles showed rates of 3-5 nmoles of NADH oxidation per mg protein per min, which was insensitive to KCN and catalase but was stimulated by 2,4-D. These membranes also showed low rates of guaiacol oxidation, but this enzyme was neither stimulated by 2,4-D nor affected by catalase as the peroxidase reaction of whole soybean cells.

Supported by N.I.H. Grant PO 1 CA-36761 to F.L.C.

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DICLOFOP RESISTANCE IN A BIOTYPE OF ITALIAN RYEGRASS

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In 1987, a biotype of Italian ryegrass (Lolium multiflorum) resistant to the aryloxyphenoxypropionic (AOPP) acid herbicide diclofop-methyl was found in a field in Oregon. We compared the growth response of resistant and susceptible biotypes to diclopfop-methyl and sethoxydim, a cyclohexandione (CHD) herbicide which has the same site of action as diclofop-methyl. At the whole plant level, the resistant biotype was tolerant to field rates of diclofop-methyl but not to sethoxydim. Acetyl-CoA carboxylase (ACCase) was extracted from etiolated leaf tissue of the resistant and susceptible biotypes and I_{50} values for AOPP acid herbicides (diclofop, haloxyfop, quizalofop) and CHD herbicides (sethoxydim, clethodim) were determined. The I_{50} values for the AOPP acid herbicides were approximately 15-fold greater in tissue extracts from the resistant biotype. There was little or no difference in I_{50} values for sethoxydim and clethodim in resistant and susceptible biotypes. The results suggest that diclofop resistance is due to a modification at the site of action (ACCase). This modification results in resistance to the AOPP acid herbicides but not to the CHD herbicides.

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INDUCTION OF GLUTATHIONE-*S*-TRANSFERASE ISOZYMES IN SORGHUM BY HERBICIDE ANTIDOTES.

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Certain chemicals referred to as herbicide antidotes protect sorghum from injury by chloroacetanilide herbicides such as metolachlor. The effect of herbicide antidotes on the glutathione-*s*-transferase (GST) isozyme complement of etiolated sorghum (Sorghum bicolor [L.] Moench) shoots was examined. Elution profiles of GST isozymes from untreated and antidote-treated seedlings were generated by FPLC-anion exchange chromatography. In untreated seedlings, there were two GST isozymes, a major isozyme active with CDNB as a substrate, and a minor isozyme which exhibited activity toward metolachlor. Treating sorghum seedlings with various antidotes resulted in the appearance of 4 to 5 additional GST isozymes (depending on the particular antidote) which exhibited activity toward metolachlor as a substrate and little or no activity with CDNB. Treating etiolated sorghum shoots with metolachlor was also found to induce at least 4 isozymes which exhibited activity toward the herbicide. An increase in GST (metolachlor) activity was detected within 4 h after treatment with 30 µM CGA-92194 but 36 h were required for maximum expression of activity. Addition of either cordycepin (3.5 mM) or cycloheximide (0.36 mM) inhibited the appearance of GST isozymes with activity toward metolachlor in CGA-92194-treated seedlings. The results are consistent with the hypothesis that antidotes protect sorghum from herbicide injury by inducing the de novo synthesis of GST isozymes which catalyze the detoxification of the herbicide.

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ENHANCED GLUTATHIONE-S-TRANSFERASE ACTIVITY IN ATRAZINE-RESISTANT VELVETLEAF

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Recently, an atrazine-resistant velvetleaf (*Abutilon theophrasti* Medik.) biotype was discovered in a field in Maryland which had been treated with triazine herbicides for at least ten years. Our previous research determined that resistance was due to enhanced detoxification of atrazine by glutathione conjugation. We compared resistant and susceptible biotypes to determine if enhanced detoxification was due to elevated glutathione (GSH) levels and/or increased activity of glutathione-S-transferase with atrazine as substrate (GST-atrazine). There was no significant differences in GSH content between the two biotypes. GST-atrazine activity was enhanced, up too nine-fold, in leaf extracts of the resistant biotype. A 24 h pretreatment with 30 uM atrazine, via hydroponic solution, did not effect GST-atrazine activity in either biotype. Purification of GST-atrazine using FPLC Mono Q chromatography showed the presence of three major isozymes in both biotypes. However, in the resistant biotype all three isozymes exibited enhanced activity. We conclude that resistance is due to enhanced activity of three GST-atrazine isozymes.

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NITROGUANIDINES INDUCE BUD BREAK AND CHANGES MEMBRANE LIPID CONTENT IN APPLE

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Bud break in apple (Malus domestica Borkh cv. Golden Delicious) was induced by 1-(3, 5-dichlorophenyl)-3-nitroguanidine or 1-(α -ethylbenzyl)-3-nitroguanidine. An increase in the galacto- and phospholipids and the ratio of the unsaturated fatty acids to the corresponding saturated fatty acids of the buds occurred as a result of induction by these two chemicals during bud break. The amounts of β -sitosterol and sitosteryl ester increased immediately after dormancy was broken and then declined. The decrease was accompanied by an increase in campesterol and stigmasterol at the beginning of rapid growth. A decrease in the ratio of free sterols to phospholipids and an increse in the ratio of campesterol + stigmasterol to sitosterol upon breaking dormancy also occurred in apple buds.

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IN VIVO CREEP TO STUDY EXTENSIBILITY AND YIELD THRESHOLD OF *PHYCOMYCES* SPORANGIOPHORES

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The pressure probe was used to conduct *in vivo* creep experiments on the sporangiophores of *Phycomyces*. Interestingly, the growth rate behavior to a step-up in turgor pressure depends on the magnitude of the step-up; small turgor pressure step-ups ($\Delta P < 0.02$ MPa) elicit an increase in growth rate, while larger step-ups elicit a decrease in growth rate. This somewhat complex growth rate behavior may be explained by cell wall strain-hardening, and subsequent adjustments of the yield threshold. (Supported by NSF Grant DCB-8801717.)

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SALINITY EFFECTS ON LEAF GROWTH, ION AND WATER RELATIONS OF KOSTELETZKYA VIRGINICA (L.) PRESL. (MALVACEAE)
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The effects of NaCl (0-255mM) on individual leaves of K. virginica, a dicot halophyte, were studied in successive harvests. Shoot growth was reduced at salinities higher than 85mM, principally from decreased leaf production and area. Salinized plants accumulated Na^+ and K^+ in their leaves and maintained a constant osmotic pressure difference between foliar and external salt concentrations. A striking leaf-to-leaf gradient in Na^+ was formed, with that ion partitioned away from the most actively growing leaves. Moreover, the Na^+ content of individual leaves remained constant or declined with time, preventing toxic build-up and early leaf death. In addition, K. virginica exhibited a strong K^+ affinity, low Na^+/K^+ ratios, and K^+ retranslocation from old to young leaves. The ability of this species to maintain favorable K^+-Na^+ relations is based in part on selective ion uptake and transport, compartmentation of Na^+ and K^+ in opposite directions along the shoot axis, and the capacity to limit foliar Na^+ loads over time.

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A NEW IN VIVO METHOD FOR DETERMINING THE NATURE OF GROWTH INHIBITION OF INTACT, SALT-STRESSED MAIZE LEAVES. GR Cramer[1], DC Bowman[1], & RY Evans[2], [1]Department of Plant Science, University of Nevada, Reno, NV, 89557; [2]Department of Horticulture, University of California, Davis, CA 95616.
Leaf elongation of maize (measured with a LVDT) is immediately inhibited by applications of salt to the nutrient solution (50 mM). Within 15 min, elongation begins to recover, but reaches a lower rate than controls. Serial applications of weight to the LVDT to create a pulling force can increase the rate of leaf elongation, but there is a threshold where no further additions of weight will increase the rate of leaf elongation in salt-stressed plants or controls. We argue that the driving force for elongation (i.e. the equivalent of P-Y) is maximal and is no longer limiting growth under these conditions. Therefore, elongation is proportional to extensibility or hydraulic conductance. Under these conditions, the maximal leaf elongation rate of salt-stressed plants is less than controls. This indicates that cell extensibility or hydraulic conductance (or both) is inhibited under salt stress. When the weights are removed from the LVDT, growth rates are identical to those before application of weights. These findings will be compared to Instron measurements.

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DYNAMIC ASPECT OF SOLUTE COMPARTMENTATION DURING ADAPTATION OF CARROT CELLS TO SALINITY. Moshe Reuveni[1], Henri R. Lerner and Alexandra Poljakoff-Mayber. Botany Department, Hebrew Univ., 91904 Jerusalem, Israel. [1]Present address: Horticulture Department, Purdue Univ., West Lafayette, IN 47907, USA.
Compartmentation of solutes between vacuole (v) and cytosol (c) during adaptation to salinity, was studied in carrot cells in suspension using the Poly-L-lysine method (Plant Physiol. 1985, 79:406-410). Carrot cells were transferred from standard medium to medium containing 150 mM NaCl. The cells plasmolyzed and 40 h later deplasmolyzed and commenced division. Solute compartmentation was assayed before transfer(A), 48 h after transfer (B), 8 days later at stationary phase(C), and 48 h after second transfer to NaCl(D). Distribution of ions changed with time of exposure to NaCl. $[K^+_v]$ decreased continuously while $[K^+_c]$ increased in B but decreased again in C&D $[K^+_v]:[K^+_c]=1$. Na^+ accumulated in both compartments, but while $[Na^+_v]:[Na^+_c]=2$ at B, at D it changed to 1, and $[Na^+_v]B:[Na^+_v]D=2$, while $[Na^+_c]B:[Na^+_c]D=1$, $[Na^+_c]:[K^+_c]$ changed from 1 at B to 2 at C&D, showing no sequestration of Na^+ in vacuole. $[Cl^-]$ accumulated in both compartments to x2 the concentration of $Na^+ + K^+$. Malate increased x2 in the cytosol in B (compared to A) but returned to situation of A at D. Free amino acids increased x5 from A to C. Adaptation of the cells to 150 mM NaCl was not accompanied by a change in size. No structural damage on submicroscopic level was noticed.

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A FLOW-THROUGH NUTRIENT SYSTEM FOR STUDY OF ROOT RESTRICTION IN *LYCOPERSICON ESCULENTUM* L.
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Root restriction reduces plant biomass, leaf area, and the number of flowers and fruits in various plants. To investigate the effects of root restriction in tomato, we have developed a flow-through system (FTS) that delivers nutrient solution to the bottom of clear plastic cylinders in which plants are hydroponically grown. This design insures adequate oxygen and nutrient delivery to active root growth. The cylinders vary in size (2.5 to 10 cm w X 5 to 20 cm h) and volume (0.025 to 1.5 l). Two cylinder sizes are used for each experiment and solutions recirculate in separate closed systems: one 50 l reservoir for each group of identical sized cylinders. Solution pH is automatically controlled to 6.0 ±0.3 pH units. The modular design of the FTS permits observation of: 1) growth of individual root systems, 2) determination of respiration and ion uptake and 3)sampling of plant tissues for metabolite and hormone assays. Preliminary testing of the FTS demonstrated a 20% reduction in root dry weight and a 40-45% reduction in shoot fresh and dry weight for the root restricted plant (using 1.5 and 0.2 l cylinders) during an eight week growth period. Data for plant growth, development, respiration and leaf and root area will be presented for tomato plants cultured in 0.025 and 1.5 l cylinders.

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P DISTRIBUTION AND ACID PHOSPHATASE ACTIVITY IN PHASEOLUS VULGARIS AND VIGNA UNGUICULATA UNDER P STRESS
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Plants of two varieties of P. vulgaris and one of V. unguiculata were grown individually in pots with river sand and watered continuously with a nutrient solution containing 1 mmol/l or 0.02 mmol/l of P. One week and three weeks after the beginning of the treatment four plants from each treatment were sampled for determination of P content and phosphatase activity in young leaves, old leaves, and roots. The activity was higher in young leaves but there were no differences between plants treated with high or low P. On the other hand, the ratio of P content in young leaves to P content in old leaves was much higher for plants grown in low P than for plants grown in high P. Apparently, the allocation of P to growing tissues did not depend on increased phosphatase activity. Instead, plants grown in low P allocated more biomass to roots, which appeared to be the primary mechanism for maintaining P uptake.

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EFFECT OF CATIONS ON THE PERMEABILITY OF PEAR LEAF CUTICLE TO PROTONS
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Direct damage to leaves by acid precipitation must involve penetration of the cuticle by the acid. We investigated how the permeability of isolated pear leaf cuticles was affected by the presence of different cations on the morphological inner side. Isolated cuticles were mounted in apparatus with the morphological outer side of the cuticle exposed to pH 3 acid and the inner side exposed to approximately pH 7 solution. Changes in the pH of the inside solution were monitored to determine cuticle permeability to protons. Permeabilities from trials with and without solutions of $CaCl_2$, $CdCl_2$, $MgCl_2$, NaCl and KCl were compared. The presence of calcium, cadmium or magnesium ions greatly reduced the permeability of the cuticles to protons. Potassium and sodium ions had no significant effect on permeability. These effects will be used to propose a modified model of proton movement through leaf cuticle.

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CHARACTERIZATION OF SINGLE AND DOUBLE GENE SODIUM CHLORIDE TOLERANT MUTANTS IN THE FERN CERATOPTERIS RICHARDII

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A strain (10α23) of the fern Ceratopteris richardii shows a high level of tolerance to NaCl (up to 275 mM) in the gametophyte generation. Genetic analysis has shown that strain 10α23 contains two unlinked nuclear gene mutations, N10 and Nα23. Pure lines containing either the N10 or Nα23 mutation show low and moderate tolerance to NaCl, respectively. Comparative studies using gametophytes and sporophytes of the wild type and mutant strains are in progress to examine the physiological basis of tolerance associated with each mutation. Initial studies of osmotic and turgor adjustment in shoots and roots of the wild type and strain 10α23 exposed to NaCl, using psychrometric pressure-volume analysis, have shown significant differences in the responses of roots, while shoots are indistinguishable.

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FAMILY VARIATION IN GROWTH RATES AND DROUGHT TOLERANCE IN BLACK SPRUCE: ADJUSTMENT OF OSMOTIC POTENTIAL AND SOLUBLE CARBOHYDRATES IN RESPONSE TO PEG-INDUCED OSMOTIC STRESS

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Black spruce (Picea mariana (Mill.) B.S.P.) full-sib families were found to differ in height growth ranking depending on soil moisture availability. Seedlings of 4 full-sib families were osmotically stressed by increasing the concentration of PEG-3350. Families did not differ in either osmotic potential or soluble carbohydrates concentrations before the stress. Osmotic potentials and monosaccharides increased significantly in all 4 families during the stress, whereas sucrose and alditols decreased. The 2 families which grew more vigorously under drought conditions, developed lower osmotic potentials, accumulated more monosaccharides and contained less sucrose than those showing lower growth rates. Thus, the degree of osmoregulation may explain differences in growth rate among families in the field where drought stress prevails.

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SPATIAL DISTRIBUTION OF ELONGATION AND SOLUTE DEPOSITION IN COTTON ROOTS UNDER SALT STRESS

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Seedlings of cotton (*Gossypium hirsutum* L.) were grown in 1/10 strength modified Hoagland nutrient solution with various combinations of NaCl and $CaCl_2$. At 1 mM Ca, 150 mM NaCl reduced overall root elongation rate to 60% of the control, while increasing Ca to 10 mM at the same NaCl concentration improved elongation rate to 80%. Analysis of the spatial distribution of elongation revealed that 150 mM NaCl in the medium shortened the growth zone by about 2 mm and also reduced the relative elemental elongation rate. The presence of 10 mM Ca at high salt condition restored the relative elemental elongation rate but not the length of the growth zone. High NaCl also significantly lowered the bulk tissue osmotic potential in the growth zone. The osmotic potential, however, was relatively uniform within the growth zone and increased only slightly in the basal regions, regardless of the NaCl concentration. The spatial distribution of solute deposition and ion densities of some elements will also be discussed.

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ISOLATION OF SALT RESPONSIVE CLONES IN BARLEY USING DIFFERENTIAL HYBRIDIZATION

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When roots of barley (Hordeum vulgare L. cv. CM 72) are grown in the presence of 200 mM NaCl for 6 d (salt-grown), the levels of a number of polypeptides change quantitatively. Quantitative changes are also evident at the mRNA level when poly(A)$^+$ RNA is translated in a rabbit reticulocyte lysate system. Based on this differential response of polypeptides and in vitro translation products to NaCl, differential hybridization was used to screen for salt responsive cDNAs. A cDNA library was constructed, using the method of Alexander (Methods Enzymol 154:41-63, 1987), from poly(A)$^+$ RNA isolated from salt-grown roots. The library contains approximately $4x10^5$ clones with 80% containing inserts. For differential screening, radioactive probes were reverse transcribed from poly(A)$^+$ RNA isolated from either control or salt-grown roots using oligo$(dT)_{15}$ as the primer. Each probe was hybridized to replica filters of the salt-grown cDNA library. Those clones which hybridized more strongly to the salt probe than to the control probe were rescreened. These clones are being characterized and the results will be presented.

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EFFECT OF SALT-STRESS ON Mg^{2-}ATPase ACTIVITY OF TOMATO ROOTS

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The Mg^{2+}-ATPase and proton transport activities of sealed plasma membrane vesicles of the domesticated tomato, Lycopersicon esculentum, were compared to those of the halophytic wild species, L. cheesmanii, in order to elucidate differences which could explain the ion transport and salt tolerance differences between the two species. Our results with the cultivated tomato indicated that salt stress did not effect directly Mg^{2+}-ATP dependent proton pumping as measured by quinacrine quenching, but that it can modulate the electrostatic properties of the plasma membrane. In order to further identify the membrane mechanisms involved in salt tolerance, we performed additional studies using the wild species. Differences between the species which can be related to the physiological mechanisms of salt tolerance will be presented.

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PREPARATION OF ANTIBODIES TO AND MICROSEQUENCING OF POLYPEPTIDES THAT INCREASE WITH SALT STRESS IN BARLEY ROOTS.

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The labeling of two polypeptide pairs with pIs of 6.3 and 6.5 and M$_r$ of 26 and 27 kD increases significantly when roots of barley (Hordeum vulgare L. cv. CM 72) are treated with NaCl. Spots containing these polypeptides were excised from Coomassie blue stained, preparative two-dimensional (2D) polyacrylamide gels to raise antibodies in mice. The mice were injected and boosted until the titers of the antisera were 1:500. The mice were again boosted, injected with a sarcoma, and the ascites fluid collected. These antibodies will be used to screen an expression library to isolate the corresponding cDNAs, to identify corresponding in vitro translation products, and to determine the subcellular location of these polypeptides. The pI 6.3 polypeptides of 26 and 27 kD were microsequenced. Preparative 2D gels were blotted to Immobilon and the spots excised from blots stained with Amido black. The N-terminal sequences were then determined using an Applied Biosystems Model 477A Pulsed Liquid Phase amino acid sequencer equipped with a Model 120A on-line PTH amino acid analyzer. The sequences for each of the pI 6.3 polypeptides were unique and differed from that for osmotin, a 26 kD polypeptide abundant in cultured tobacco cells adapted to high levels of NaCl. Oligonucleotides corresponding to these microsequences will be used as probes to obtain cDNAs for the polypeptides.

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P UPTAKE AND TRANSLOCATION: EFFECT OF SALINITY AND CA

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Cotton seeds (Gossypium hirsutum L. cv Alcala SJ-2) were grown 10 to 12 days in a complete nutrient solution containing 1 mol m^{-3} Ca^{++} and 10 mmol m^{-3} Pi. Additions were made of NaCl to 150 mol m^{-3} and $CaCl_2$ to 10 mol m^{-3} for the high salt and Ca treatments respectively.

The inhibiting effects of salinity commence as early as two hours after salinization with an inhibition of both ^{32}P uptake and translocation to the shoot. After a few days, a marked increase in ^{32}P translocation to the leaves of the salinized plants is observed relative to the control, due in part to dilution by growth in the control plants. Increasing the Ca^{++} concentration in the nutrient solution partially relieves (35%) the inhibition of shoot growth over several days, while the inhibition of 32P translocation is relieved 30% within 2 h.

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HIGHER TREHALOSE ACCUMULATION IN RHIZOBIA UNDER SALT STRESS

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Trehalose is a major sugar synthesized by Rhizobium spp. in legume nodules, and is the sole sugar found in cultured rhizobia. Trehalose appears to have an osmo-protective role in other organisms, so we investigated the effect of salt on the trehalose pool in rhizobia grown in defined media. Soluble carbohydrates were extracted with EtOH and analyzed by gas chromatography. Three of 7 R. leguminosarum biovar phaseoli strains tested had up to 5X higher trehalose levels in salt stressed cells. The same response was obtained with 4 different C sources and with KCl instead of NaCl used to induce osmotic stress. The trehalose pool increased with increasing NaCl concentration up to 100 mM NaCl. During the growth cycle, trehalose levels increased during log phase and rapidly decreased at stationary phase. Several other rhizobial species were tested, but only R. loti accumulated more trehalose in response to salt stress. Because this response is not uniform among rhizobia, it appears unlikely that trehalose accumulation is a general mechanism for adaptation to salt (or osmotic) stress in Rhizobium spp.

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THE EFFECT OF NACL SALINITY ON BELL PEPPER PHOTOSYNTHESIS

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Plants exposed to saline conditions often photosynthesize at reduced rates. To better understand how these reductions occur, gas exchange measurements on attached leaves of bell pepper (*Capsicum annuum*) were made throughout a two week period of salinization. Plants were grown in solution culture and NaCl concentration was increased by 25 mM per day to 50, 100 or 150 mM. Measurements were made at 70-1900 $\mu l.l^{-1}$ external CO_2 and 1800-1850 $\mu E.m^{-2}.s^{-1}$ PPFD. The older leaves, on which gas exchange was estimated, remained healthy in appearance. Rates of CO_2 exchange remained high in unsalinized controls and at 50 mM NaCl, but were reduced significantly at 100 and 150 mM NaCl. Stomatal conductance decreased with the increases in salinization and with time of exposure, yet calculated substomatal CO_2 levels were not reduced. Therefore stomatal closure did not decrease photosynthesis. Instead, assimilation rates at a given substomatal CO_2 concentration were depressed and also decreased with time. The rate of CO_2 exchange in stressed plants did not reach a clear maximum, even at the highest external CO_2 concentration. Exposure of plants to 150 mM NaCl often increased the CO_2 compensation point. If photosynthesis at low CO_2 concentrations is regulated primarily by Rubisco, then the data indicate a reduction in either the amount of this enzyme or its affinity for substrate. Xylem water potentials also decreased with salinization, but the effect of this on photosynthesis, under these conditions, is not known.

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SALINITY RESPONSES OF GROWTH AND CARBON METABOLISM IN SUNFLOWER

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The effects of salinization from 10mM to 100mM NaCl were examined in sunflower (*Helianthus annus*) plants, subjected to both early (before the 2nd leaves began expansion) and late (after the 5th and 6th leaves began expansion) salinization. Over the next two weeks, there was an overall reduction in leaf area and dry weight, an increase in stem and petiole weight, and a decrease in tap-root weight in more saline conditions; there were no differences in leaf initiation rates.

Photosynthetic rates were measured for leaf disks using an O_2 electrode and for individual leaves under their growth conditions using infra-red gas analysis. The light response curves of leaves from the two treatments were similar with quantum yields slightly higher in the salinized leaves. *In vivo* net fixation rates and stomatal conductances were similarly unaffected by salinity.

Carbohydrate analyses showed a tendency toward increased hexose levels with salinity; sucrose was constant with age and salinity. With the exception of recently expanded leaves, the starch concentrations were not affected by salinity, and in all cases, more than 80% of the starch was mobilized during the night. These results showed that availability of carbon was not the cause of salinity induced growth reductions.

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STUDIES ON PROLINE ACCUMULATION FOLLOWING SALT STRESS IN CHLORELLA 580. Mary Bodnar, Paul Behrens, and Scott Bingham. Martek Corporation, 6480 Dobbin Rd., Columbia, MD 21045. Chlorella 580 has been reported to accumulate large quantities of proline after addition of 1M NaCl to the growth medium (Leavitt, R. 1986 Nova Hedwigia 83:139). We have confirmed these results by finding that the organism synthesizes proline to 18% of its dry weight, reaching a maximum level 4-5 days after salt addition. We have assayed, in vitro, the activity of glutamate kinase, the first enzyme in the committed proline biosynthetic pathway. Specific activity of glutamate kinase increases ca 3 fold by 24 hours following salt addition, suggesting that additional enzyme has been synthesized or existing enzyme has been activated. Glutamate kinase, in most organisms, is feedback inhibited by proline, providing end product regulation of the pathway. However, the activity of the enzyme from non-salt stressed Chlorella 580 is insensitive to inhibition up to at least 100mM proline. These results suggest a novel regulation of the proline pathway in Chlorella 580. Further studies are underway to elucidate the mechanism of this regulation.

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GROWTH OF INTACT MAIZE PLANTS IN RELATION TO pH, AND CHANGES IN DIURNAL PROTON EFFLUX FROM THE ROOTS.

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Growth and proton release from intact maize plants (cv. Pioneer 3906) were measured in relation to pH and uptake of NH_4^+ and NO_3^- by use of batch cultures, and by continuous flow cultures equipped with pH-stat's. In young plants uptake of NH_4^+ and release of protons were reduced at pH 4.0, but young plants assimilated more NO_3^- than NH_4^+ at pH 6.0 and 6.5 while older plants assimilated more NH_4^+ than NO_3^-. Young seedlings were most active in proton extrusion during the night, but after 7-14 days this pattern changed and plants released more protons during the light phase. A possible ontogenetic change in the diurnal cycle of proton extrusion linked with changes in the preference for uptake of NH_4^+ and NO_3^- will be discussed.

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DEPENDENCE OF MAGNESIUM TRANSLOCATION ON PHOSPHORUS SUPPLY

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The role of phosphorus (P) in leaf magnesium (Mg) nutrition and photosynthesis was investigated in field and glasshouse experiments with grapevine. Under low soil P conditions, leaf photosynthesis (approx. 0.7 nmol cm^{-2} s^{-1}) was limited by leaf Mg nutrition. When P was applied to soil, leaf P and Mg and the rate of photosynthesis increased. When Mg was applied as a foliar spray, leaf Mg but not P increased and photosynthesis increased to the same rate observed following P treatments (approx. 1.0 nmol cm^{-2} s^{-1}). When vines were grown in the glasshouse without P, Mg accumulated in large roots to 2x the concentration in roots of +P vines. Analysis of xylem sap showed that Mg in +P vines was 2x greater than in -P vines. The results show that Mg uptake by roots is not sensitive to P supply and that the translocation of Mg from roots to shoots is highly dependent upon P supply to the roots.

709

ELECTROPHYSIOLOGICAL EVIDENCE FOR THE ASYMMETRIC CHARACTER OF CUTICLES

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The mechanism of ion permeation through leaf cuticles was investigated by probing a polar pore model postulated by others to account for the permeation of small nonelectrolytes. Diffusion potentials of KCl were measured across cuticles isolated from leaves of three tree species. Diffusion potentials were asymmetric, as opposed to the symmetric values calculated by the Goldman Equation. Two artificial membranes that generated symmetric diffusion potentials, but with different magnitudes of charge, were placed together as a bilayer. This created a physical model that simulated a cuticle by generating asymmetric diffusion potentials, and enabled us to develop a charged pore model for the permeation of ions across cuticles.

710

DOES ETHYLENE MEDIATE WATER-STRESS-INDUCED MALE STERILITY IN WHEAT?

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Water stress during meiosis in anthers induces male sterility in wheat. The mechanism of this response remains obscure, although some evidence favors a role by ABA, and ethylene has been suggested as another mediator. In the course of determining the involvement of ethylene in stress-induced male sterility, we found that application of ethephon (2-chloroethylphosphonic acid) can partially mimic the effects of water stress on male fertility. However, durations of the stages of maximum sensitivity to ethephon and water stress appear to be somewhat different. There are side effects of ethephon concentrations high enough to induce sterility levels similar to those caused by stress. Preliminary experiments with inhibitors of ethylene synthesis and action suggest that the effects of water stress on male fertility may not involve endogenous ethylene. Results of further experiments to critically examine this hypothesis will be presented.

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THE INTERACTION OF ETHYLENE AND POLYAMINE METABOLISM DURING OZONE STRESS OF TRITICUM AESTIVUM

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Boyce Thompson Institute, Cornell University, Ithaca, NY 14853

We previously reported that wheat seedlings respond to ozone, in part, by inducing ethylene production and altering polyamine metabolism. Little is known about the interaction between these two closely associated pathways in vivo. During a 7 day time course, ethylene production was initiated in intact seedlings within the first 4 h (120 ppb, 8 h/day) ozone exposure. Ozone induced ethylene production fluctuated throughout the time course, declining each night and rising each morning within the first 4 h of ozone exposure. Alteration of polyamine metabolism only occurred after several days of ozone exposure. This pattern of polyamine alteration also occurred when amino acids (mM) were applied to the basal internode of intact wheat seedlings. Ethylene production, however, was not induced with amino acid application. This suggests that interaction between polyamine and ethylene metabolism within an intact plant is not tightly coupled. Changes in common precursors (e.g. S-Adenosyl Met.) to both pathways during ozone exposure will be discussed.

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REGULATION OF ROOT/SHOOT RATIOS BY WATER DEFICIT AND ABSCISIC ACID (ABA) TREATMENT

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In higher plants, shoot growth rate and tissue water potential decline as a water deficit develops. However, at some tissue water potentials where shoot growth is inhibited, roots continue to elongate. While the adaptive value of this response is clear, the molecular basis of differential growth inhibition is unknown. We examined hypocotyl/root growth, polysome status, and ABA content in etiolated soybean seedlings whose growth was inhibited by water deficit or exogenous ABA. Both treatments affected growth and dry weight in a similar fashion. Maximum inhibition of hypocotyl growth occurred when internal ABA levels (modulated by ABA application) reached the endogenous level found in water deficient seedlings. Root growth was unaffected by water deficit and most ABA treatments (in some, growth was promoted). In every seedling section examined, ABA content increased approx. 10-fold in water deficit. These data suggest that root/shoot ratios could be modulated by differing sensitivity to ABA. Changes in polysome status and polysome RNA translation products will be described.

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ABSCISIC ACID DIFFERENCES IN SOYBEAN AND SUNFLOWER LEAVES DURING WATER STRESS

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Soybean (Glycine max. (L.) merr. cv Amsoy 71) and sunflower (Helianthus annuus L. cv S-1888) were grown in the same pot in growth chamber. Water stress was employed at 35 days of age by withholding water. The relationships of abscisic acid (ABA) and photosynthesis during water stress was investigated by comparisons of endogenous ABA and some photosynthetic parameters between the two species. ABA was quantitatively analyzed by GC-MS. It was found that ABA increased dramatically as desiccation continued for both species; however, ABA was 3.2 fold higher in soybean than in sunflower at both d 4 and d 5 of stress, and reached 2.3 mg/g fw before rewatering. The much higher ABA contents in soybean was associated with lower stomatal conductance, transpiration rate and CER. After rewatering, although ABA decreased rapidly, soybean had consistently higher ABA level with lower photosynthetic recovery, stomatal conductance and transpiration than sunflower, whereas ABA in sunflower had almost returned to predesiccation level at 9 h after rewatering. It was concluded that one of the differences in photosynthesis between the two species was due to difference in ABA contents, at least in water stress condition.

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PRESSURE-VOLUME RELATIONS OF ECHINCHLOA TURNERIANA, ECHINOCHLOA CRUS-GALLI AND PENNISETUM AMERICANUM

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Echinochloa turneriana is a wild grass native to the arid Channel Country of inland Australia. Its drought resistance was compared to that of Pennisetum americanum and Echinochloa crus-galli var. oryzicola. Plant water relations were determined by the pressure-volume method. Active osmotic adjustment under drought was detected in P. americanum, but not in the Echinochloa species. The bulk modulus of elasticity decreased significantly under drought in E. turneriana, but not in the other two species. Once water deficits developed, turgor was significantly decreased in E. turneriana despite a decrease in the bulk modulus of elasticity. The results support the view that E. turneriana's rapid development is its most important adaptive feature, allowing the plant to complete its life cycle before the onset of a severe water deficit.

715

WATER-STRESS INDUCED ALTERATIONS IN GROWTH AND ESSENTIAL OIL ACCUMULATION IN PEPPERMINT (*Mentha x piperita* L.)

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The effect of plant water stress on the accumulation of essential oils in peppermint leaves was examined from greenhouse experiments in which hydroponically grown peppermint was subjected to one and two weeks of three levels of water stress (solution osmotic potential of 0, -2, -4 and -6 MPa) induced by PEG. Plants were maintained for one week in the nutrient solution prior to induction of water stress. As stress increased, leaf area, and leaf and stem dry weight decreased; and essential oil (EO) content increased (%g dry wt). Leaves from the highest stress treatment (-6 MPa) exhibited greatest increase in EO content (7.1%) compared to plants of -4 MPa (5.4% EO), -2 MPa (5.2% EO) and the non-stressed control (0 MPa) with 4.4% EO. Water stress induced qualitative changes in EO composition. Increasing stress led to increases (relative percent) in menthone, menthofuran, piperitone, sabinene and sesquiterpenes; and decreases in 1,8-cineole, pulegone, menthylacetate and β-cubebane.

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TOLERANCE TO LOW PHOSPHORUS LEVELS IN AN ANTHOCYANINLESS TOMATO VARIETY (*LYCOPERSICON ESCULENTUM*)

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Two varieties of tomato (*Lycopersicon esculentum*) with varied responses to low phosphorus (P) levels were grown in hydroponic culture where phosphorus concentrations varied from 2mM to 0.03mM. Previous observations suggested that Heinz variety (var) 883 was much less tolerant to low P than an antocyaninless var 957. Seeds were germinated in vermiculite moistened with distilled water then transferred to hydroponic media 18 days after germination. The plants were observed daily and the fresh weight of each plant was taken weekly. After five weeks, photosynthetic rates (of leaf 6 and 10), dry weights and the mineral content of the plants were measured. At each fresh weight measurement, var 957 showed increased growth over var 883 in every concentration of phosphorus. Dry weights also reflected this trend. Var 957 showed lower net photosynthetic rates than 883 at all but the lowest phosphorus concentrations (0.03mM). The anthocyaninless variety also contained less phosphorus in its tissues than did 883. 957 exhibited none of the typical phosphorus stress symptoms such as purpling of the leaves or diminished foliage while both of these characteristics were prominent in var 883.

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ROOT RESPIRATION AND NH_4^+ AND NO_3^- ABSORPTION IN RELATION TO ROOT HYPOXIA IN THE WILD VARIETY OF TOMATO, *LYCOPERSICON ESCULENTUM* VAR. *CERASIFORME*.

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Hypoxic conditions may limit root respiration and nitrogen absorption by plant roots, yet little is known of the relative sensitivity of these processes or their interdependence. Using a steady-state nutrient and gas-exchange system, we monitored O_2 uptake, CO_2 evolution and net NH_4^+ and NO_3^- uptake by roots of intact plants of two accessions of the wild variety of tomato, *Lycopersicon esculentum* var. *cerasiforme*. Roots were exposed to a series of decreasing solution O_2 concentrations ranging from 250 to 25 μM, with N provided as either 200 μM KNO_3, 200 μM NH_4Cl, or 100 μM KNO_3/NH_4Cl. O_2 uptake was inhibited more by low O_2 concentration than was CO_2 release, and NH_4^+ uptake was inhibited more than NO_3^- uptake. The tomato accession from frequently-flooded habitats showed less inhibition of respiration and uptake of both N forms than the accession from well-drained soils. The possibility that NO_3^- may serve as an alternate electron acceptor under hypoxia is discussed.

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EFFECT OF GLUTATHIONE ON PHYTOCHELATIN SYNTHESIS AND CADMIUM TOLERANCE IN TOMATO CELLS

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Phytochelatins (PCs, poly (γ-glutamylcysteinyl)glycines) are a family of peptides synthesized by plants in response to cadmium, and bind cadmium within the cell. Production of PCs is accompanied by depletion of GSH which is a substrate for PC synthesis. Inhibiting the synthesis of GSH with buthionine sulfoximine (BSO) blocks production of PCs in response to cadmium, increases the cellular concentration of free cadmium, and reduces cadmium tolerance. In cells treated with BSO, the addition of GSH to the medium restores both the synthesis of PCs and tolerance to cadmium. To determine if the supply of GSH affects the rate of PC production, cells were exposed to 150 μM CdCl2 in the presence of 0 to 500 μM GSH. After 6 hours, cells treated with 500 μM GSH contained three times the amount of PCs found in cadmium treated cells without GSH. When the levels of individual PCs were examined, GSH treatment was found to stimulate the production of PC2 and PC3 but have little effect on the level of PC4. These results indicate that availability of GSH affects the rate of PC synthesis. The enzyme(s) that assembles PCs is not processive and substrate concentrations (GSH or preformed PCs) determine which PCs are formed.

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D-ASPARAGINE STIMULATES SALT TOLERANCE IN *ARABIDOPSIS THALIANA*.

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The effectiveness of exogenous *L*- and *D*-asparagine in mitigating NaCl inhibition of *'Columbia' Arabidopsis thaliana* seed germination was assessed under aseptic conditions. Inhibition of germination by NaCl concentrations below 225 mM was substantially reversed by the addition of *L*- and *D*-asparagine to the medium. Maximal reversal of NaCl inhibition occurred between 2 to 4 mM of both *L*-and *D*-asparagine. Higher concentrations of NaCl prevented seed growth whether asparagine was present or not. Total Na^+ uptake or K^+ efflux by whole seedlings whose salt tolerance was stimulated by exogenous asparagine was similar to seedlings receiving an equivalent amount of NaCl without asparagine. Supported by USDA Special Grant No. 88-34186-3340, Southwest Consortium of Plant Genetics and Water Resources.

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NUTRIENT DYNAMICS IN COWPEAS AS INFLUENCED BY NUTRIENT SUPPLY AND PLANTING DENSITY

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The dynamics of nutrient distribution in plant tissues as influenced by culture conditions has been little studied in cowpeas. Vigna unguiculata (L.) Walp. cv. California Black-eye plants were grown in a 5 x 5 study of nutrient supply and planting density. Plants were harvested early in the reproductive phase, divided into leaf, stem, root, petiole, and flower and pod components and analyzed for 9 elements by AA spectroscopy. Some elements (K,P) decreased in tissues as nutrient supply decreased, while others (Ca,Mn,Na) increased in tissues with decreasing supply. A third group of elements (Mg,Fe,Zn,Cu) showed no change, or a non-linear change in tissue concentration in relation to supply. These patterns allow analysis of nutrient translocation, and nutrient distribution in the whole plant. Tissue nutrient concentration seems to be more strongly regulated by nutrient supply than by planting density.

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A STUDY OF MECHANISMS OF AL^{3+} TOXICITY AND TOLERANCE IN WHEAT (TRITICUM AESTIVUM) ROOTS.

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Yield reduction of most agricultural crops in acid soils is correlated with increasing concentrations of free aluminum in the soil solution. In wheat (Triticum aestivum), Al^{3+} accumulates in the root tips inhibiting their growth. Two-dimensional gel electrophoresis analysis of proteins isolated fom the root tips indicates that Al^{3+} induces changes in the accumulation of soluble proteins in both susceptible and tolerant wheat cultivars (Victory and Atlas 66, respectively) after 24h of Al^{3+} treatment at concentrations that inhibit root growth. To determine whether or not these proteins are accumulated as a result of gene activation, RNA will be extracted from the tips of control and treated roots and translated in vitro. The translated proteins will be analyzed by 2-D gel electrophoresis. Possible mechanisms of Al^{3+} tolerance and toxicity in wheat will be discussed.

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ANALYSIS OF PROTEINS ASSOCIATED WITH ALUMINUM TOXICITY IN WHEAT ROOTS USING 2-D PAGE AND COMPUTERIZED IMAGE PROCESSING

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Experiments were conducted to determine if aluminum toxicity causes specific changes in the pattern of protein synthesis in wheat root tips, and if these changes are correlated with tolerance to Al in different cultivars. Proteins from cytoplasmic and microsomal fractions were separated by 2-D PAGE. Computerized image processing was used to enhance gel images, improve gel-to-gel registration, and quantify differences between composites of replicate gels.

The primary response to Al toxicity was the enhanced formation of a subset of 8-10 cytoplasmic and 3-5 microsomal proteins, all of which were common to the 4 cultivars examined, including Al-sensitive Victory and Al-tolerant Atlas. Exposure to Al did not result in formation of any major proteins unique to tolerant cultivars. These results do not support the idea that inducible proteins mediate variable tolerance to Al in wheat cultivars.

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CADMIUM-SULFIDE CRYSTALLITES IN CD-γ-GLUTAMYL PEPTIDE COMPLEXES FROM *LYCOPERSICON* AND *DAUCUS*

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Hydroponically-grown tomato plants (*Lycopersicon esculentum* P.Mill. cv stone) and suspension-cultured carrot cells (*Daucus carota* L.) exposed to 100 μM cadmium salts produced metal-γ-glutamyl peptide complexes containing acid labile sulfur. The properties of the complexes resemble the Cd-γ-glutamyl complexes from *Schizosaccharomyces pombe* and *Candida glabrata*, known to contain a CdS crystallite core. The crystallite core is stabilized by a coating of peptides of the general structure $(\gamma\text{-Glu-Cys})_n\text{-Gly}$. The Cd-peptide complexes contain predominantly peptides of n_2, n_3, n_4 and $n_3$desGly. Zn-peptide complexes were also isolated from carrot cultures grown in MS medium supplemented with 2 mM Zn and cysteine. Results of preliminary characterization of these complexes are consistent with the presence of a colloidal particle similar to that of the Cd-complexes.

724

THE KINETICS OF ALUMINUM ACTION ON THE ELONGATION OF PRIMARY ROOTS OF ZEA MAYS

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Aluminum (Al) is a phytotoxic element that inhibits root elongation. We used a root auxanometer to examine the kinetics of Al action on primary roots of maize (cv Merit) growing in oxygenated 0.4 mM $CaCl_2$ adjusted to pH 4.5. One mM Al promoted elongation almost immediately (< 2 min). Within 30-45 min the rate of elongation began to decline and decreased to zero within 36-45 h. Exposure to 10 μM Al also promoted elongation almost immediately, however, the new steady rate was achieved only after about 25 min. When roots were decapped prior to exposure to 10 μM Al, elongation was still promoted but the new steady rate of elongation was not attained until 1.5 h after addition of Al. The results indicate that Al initially promotes root elongation and that the root cap is important for rapid responses to Al.

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RATE OF PHYTOCHELATIN PRODUCTION: IMPORTANCE TO METAL TOLERANCE

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Low molecular weight, cysteine-rich metal-binding peptides are produced in plants exposed to metals. This study focuses on the hypothesis that the rate of phytochelatin production is important to metal tolerance. Four clones of *Deschampsia caespitosa* tolerant of Cu and Ni, and one non-tolerant clone, were exposed to Cu and Ni for various times up to 24h. Root extracts were chromatographed on an anion exchange column to separate the metal-peptide fraction from unbound metal. In three metal-tolerant clones up to 95% of the buffer-soluble Cu and Ni was found in the metal-peptide fraction after 1h of exposure, with little change occurring subsequently. The non-tolerant clone bound only 2% of the metal after 1h. Copper remained low over 24h while Ni increased in the peptide fraction to the levels found in the tolerant clones. One metal tolerant clone did not conform to the patterns found in the other three. These data support the hypothesis that metal tolerance is, in some cases, related to the ability of roots to rapidly bind the metal that enters cells.

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PSYCHROMETRIC PRESSURE-VOLUME ANALYSIS OF RAPIDLY EQUILIBRATING TISSUES

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A multi-chambered thermocouple psychrometer was used to construct pressure-volume (PV) plots for tissues whose size, fragility or absence of stalk precluded use of the pressure chamber. Embryogenic *Dactylis* callus, *Ceratopteris* sporophytes and roots of various species were examined under either non-stress or saline conditions. We obtained consistent and reasonable estimates of typical PV parameters, including symplastic water fraction and component water potentials, water contents, and elastic moduli at and between full and zero turgor. Standard errors for these parameters and correlation coefficients of the zero-turgor regression lines (3-7 pairs) compared favorably to those commonly obtained in the traditional PV analysis of shoot tissues using the pressure chamber. Full turgor osmotic potentials of non-stressed roots of *Ilex*, *Lycopersicon* and three *Rosa hybrida* cultivars ranged from -0.72 to -0.91 MPa. An adjustment of 0.72 MPa (-0.61 to -1.33 MPa) in full turgor osmotic potential was observed in *Ceratopteris* sporophytes exposed to 60 mM NaCl for ten days. Osmotic potential at harvest of *Dactylis* callus grown for three weeks on 0, 50, 100 and 150 mM NaCl was -0.76, -0.90, -1.49 and -1.61 MPa, respectively. As equilibration of samples with the gas phase within the psychrometer chambers is vital in acquiring accurate estimates of water potential, rapid equilibration times (less than 30 minutes) are necessary to obtain a sufficient number of points to construct the plots. Theoretically, the technique may be used to characterize osmotic and turgor regulation in any sparsely or non-cuticularized tissue having a fairly high surface area-to-volume ratio.

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ENHANCEMENT OF WATER STATUS, GROWTH, AND YIELD OF TRANSPLANTED BELL PEPPER SEEDLINGS BY USE OF AN ANTITRANSPIRANT Peter Nitzsche, Gerald Berkowitz, and Jack Rabin Horticulture and Coop. Extn. Dept., Rutgers Univ., New Brunswick, NJ 08903

Field studies indicate that leaf water potential (Ψw) depression for days, or even hours, can negatively affect subsequent growth and yield of transplanted seedlings. Studies were undertaken to ascertain the usefulness of a wax emulsion antitranspirant for overcoming this transplant shock. Screening studies indicated that a wax emulsion (Folicote) was only effective as a antitranspirant when combined with certain surfactants. Relative phytotoxicity of the screened surfactants was ascertained by monitoring ethylene evolution from detached, water stressed leaves. A non-phytotoxic wax emulsion/spreader-sticker surfactant formulation was tested in field studies for antitranspirant effectiveness. In treated plots, leaf resistance immediately after transplanting was increased, resulting in Ψw increases of 0.45 megapascals for 7 d. This enhanced seedling water balance resulted in a reduction in initial leaf abscission, and significantly greater leaf area throughout the growing season. Enhanced seedling growth in treated plots resulted in a significant 25% increase in fruit yield.

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THE PROTOPLAST VOLUME:WATER POTENTIAL RELATIONSHIP AND BOUND WATER FRACTION IN SPINACH LEAVES Santakumari Mane & Gerald Berkowitz Hort.Dept., Rutgers U., New Brunswick, NJ 08903

Methods used to estimate the (non-osmotic) bound water fraction (BWF) (i.e., apoplast water) of spinach leaves were evaluated. Studies using three different methods of pressure/volume (P/V) curve construction all resulted in a similar calculation of BWF; 40%. The theoretically-derived BWF, and the water potential (Ψw)/relative water content relationship establlshed from P/V curves were used to determine the relationship between protoplast (i.e. symplast) volume and Ψw. Another method of establlshing the protoplast volume/Ψw relationship in spinach leaves was compared with the results from P/V curve experiments. This second technique involved the vacuum infiltration of solutions at a range of osmotic potentials into discs cut from spinach leaves. These solutions contained radioactively labelled H_2O and sorbitol. This dual label infiltration technique allowed for simultaneous measurement of the total, and apoplast volumes in leaf tissue; the difference yielded the protoplast volume. The dual label infiltration experiments and the P/V curve constructions both showed that below -1 megapascals, protoplast volume decreases sharply with decreasing water potential; with 50% reduction in protoplast volume occurring at -1.8 megapascals leaf water potential. Supported by NSF grant DMB8706240.

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HYDRAULIC CONDUCTIVITY & VESSEL ANATOMY OF ROSES Alan Darlington & Michael Dixon Dept. of Hort. Science, Univ. of Guelph, Guelph, Ont., Canada, N1G 2W1

Two major regions of low conductivity were found in rose explants; 1) the abscission zone between the peduncle and the stem and 2) the peduncle directly below the ovary. The section of peduncle between these regions of low conductivity offered less resistance to flow but still maintained a lower conductivity than the region of stem below the peduncle. The zone of low conductivity below the ovary corresponds to the area where earlier anatomical work had found a reduced number of vessels as well as a decrease in vessel diameter. These two factors could lead to the decrease in conductivity seen. Contrary to this, the vessels passing through the abscission zone showed neither a decrease in numbers nor size. It was found that all vessels of the stem ended prior to passing through the abscission zone. Thus the decrease in conductivity was due to these end walls. Implications of this will be discussed as they pertain to water relations considerations.

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CHANGES IN SHOOT WATER POTENTIAL CHARACTERISTICS OF NEWLY PLANTED TSUGA HETEROPHYLLA AND THUJA PLICATA SEEDLINGS. Steven C. Grossnickle[1] & James T. Arnott[2];[1] British Columbia Research Corporation,3650 Wesbrook Mall,Vancouver,B.C. V6S 2L2; [2]Forest Canada, 506 W. Burnside Rd.,Victoria,B.C. V8Z 1M5

During plantation establishment, species specific response to water stress is critical for survival and growth. Tsuga heterophylla and Thuja plicata seedlings were planted in both a semicontrolled field (optimal soil moisture)and a reforestation site. Diurnal (shoot water potential,stomatal conductance,and photosynthesis)and seasonal (pressure-volume analysis) patterns were monitored. Tsuga heterophylla seedlings on the reforestation site had more stressful diurnal physiological patterns which resulted in osmotic adjustment and higher maximum bulk modulus of elasticity throughout the entire growing season. For T.plicata; similar diurnal physiological stress patterns resulted in osmotic adjustment only in June for seedlings planted on the reforestation site. Seasonal patterns of maximum bulk modulus of elasticity for T.plicata were one-half those of T.heterophylla. This difference may contribute to species specific seasonal drought tolerance strategy.

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STOMATAL INHIBITION OF PHOTOSYNTHESIS UNDER DROUGHT STRESS IN SOYBEAN J. R. Frederick, D. M. Alm, R. R. Wise, J. D. Hesketh, & F. E. Below Dept. of Agronomy and USDA-ARS, Univ. of Illinois, Urbana, IL 61801

Afternoon CO_2-exchange rates (CER) of field-grown soybean [Glycine max (L.) Merr.] were measured on upper leaves supplied with either an ambient (340 µl/L) or high (1800 µl/L) concentration of CO_2 to determine the influence of stomatal closure under drought stress on leaf photosynthesis. The cultivars Williams 82 and Asgro brand 3127 were drought stressed throughout reproductive development, with irrigated control plants grown adjacent to the drought-stressed plots. Over both cultivars, drought stress decreased seed yield and maximum leaf area by 48 and 31%, respectively. During seed development, afternoon leaf water potentials were decreased by drought an average of 0.31 and 0.23 MPa for A3127 and Williams 82, respectively. With ambient CO_2 concentrations, the average decrease in afternoon CER due to drought stress was 53% during seed fill, whereas a 2.1-fold average increase in stomatal resistance was observed. In contrast, internal CO_2 concentrations were similar between soil moisture treatments. Using high CO_2 concentrations, CER were similar or higher under drought stress than under irrigation for both cultivars. These data suggest that stomatal closure may be the primary limiting factor of soybean photosynthesis under slowly-developing drought stress.

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DROUGHT STRESS RESPONSES OF MICROSERIS SPECIES DIFFERING IN NUCLEAR DNA CONTENT

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Reduced DNA content is hypothesized to be of an adaptation to stressed habitats. Responses to drought stress were compared in two Microseris species (M. bigelovii, DNA = 2.6 pg nucleus^{-1}, more xeric; M. laciniata, DNA = 6.8 pg nucleus^{-1}, mesic). Mesophyll cell volume was positively correlated with DNA content and negatively correlated with tissue elasticity, i.e. low ε and thin cell walls. Drought stress increased leaf tissue elasticity (lower ε, thinner cell walls). Cell volume, cell wall thickness, cell number and leaf area were decreased most by drought stress in M. laciniata. Osmotic adjustment was observed in both species. Solute contribution to the change in ψ_s was larger in M. bigelovii. This indicates that Microseris species respond to low water availability by maintaining ψ_p with: (1) small cell volumes, (2) elastic tissues and (3) osmotic adjustment.

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EFFECTS OF WATER DEFICIT ON CELL SIZE IN GROWING REGIONS OF CORN ROOTS

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Roots growing under water stress commonly exhibit a marked decrease in growth rate and in diameter. We have investigated the cellular growth patterns underlying these phenomena. Corn roots were grown in either saturated vermiculite or at a moisture level that was 2% of the control. Median longitudinal sections of fixed roots were examined. The cell lengths in the shortened elongation zone of water stressed roots were greater than those of cortical cells in the comparable location of well-watered roots. Nearly two-fold differences in cell length were seen in the region 3-4 mm behind the root apex. The shortened growth zone does lead to a final mean cortical length approximately 30% shorter in the stressed roots. These differences were present regardless of the final length of the control roots. This data, and the much slower growth rate seen in these water stressed roots suggests that the water deficit causes a significant reduction in the rate of cell supply to the cortical cell files.

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EFFECT OF WATER STRESS ON EARLY KERNEL DEVELOPMENT IN MAIZE.

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Water stress during early grain development in maize causes a greater reduction in kernel weight in apical than in basal regions of the ear, but the mechanism of this effect is not known. Water was partially withheld from plants for two weeks during the endosperm cell division and early storage product accumulation phases. Cell numbers, hormone levels, activities and the relative mRNA transcript abundance of ADPG starch synthase, sucrose synthase, and a zein were studied in kernels from different ear regions. Endosperm from apical kernels in stressed plants had lower cell numbers than controls. ADPG starch synthase activity was lower in apical than middle kernels of both control and stressed plants. Significantly less message for sucrose synthase was present in stressed than control apical endosperms; in middle kernels there was a delay in sucrose synthase mRNA accumulation in stressed plants. Zein mRNA accumulation was slightly lower and delayed in apical endosperm of stressed plants, while in basal regions zein mRNA was higher in stressed than control plants. Water deficit may decrease kernel mass by initially decreasing cell division, which in turn may decrease the expression of activities associated with storage product accumulation.

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RESPONSE OF CULTURED MAIZE KERNELS TO OSMOTIC STRESS

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Maize is sensitive to water stress during early kernel development when endosperm cell division is occurring, resulting in fewer endosperm cells and reduced grain dry weight. Previous work indicated that kernels cultured in medium containing elevated levels of ABA respond in a similar way. To further understand the way in which kernels respond to stress, maize kernels were cultured in media of three osmotic potentials (-1.0, -1.3 or -1.8 MPa) using 10, 15 or 20% (w/v) sucrose with or without addition of sorbitol. After 5 d in culture (10 d after pollination), endosperm fresh weights were similar in all treatments except for those cultured in 20% sucrose medium (-1.8 MPa). In this case, endosperm fresh weights were reduced by 50%. At osmotic potentials less than -1.0 MPa (10% sucrose) the number of cells per endosperm was reduced and endosperm ABA concentrations were increased. No differences were detected in endosperm glucose concentrations, but an increase in endosperm sucrose concentration was apparent with decreasing osmotic potential of the medium. Lowering the osmotic potential of the medium, regardless of solute, resulted in increased endosperm ABA and sucrose concentration, and decreased endosperm cell number.

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EVALUATION OF CONTRASTING CELLULAR-LEVEL DROUGHT ACCLIMATION RESPONSES

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Three contrasting metabolic adaptions to low water potential (Ψw) are osmotic adjustment (OA), increased wall elasticity (E), and increased bound water fraction (BWF). All three mechanisms result in the maintenance of turgor at lower cell Ψw. However, increased BWF causes a greater rate of decline in relative water content (RWC) as Ψw declines, and OA typically causes a decrease in E at high RWC. It has been shown that OA causes RWC to decline less at low Ψw, resulting in the maintenance of greater protoplast volume. Enhanced photosynthesis at low Ψw has been associated with this volume maintenance. Three varieties of Triticum which differ in their response to stress in terms of OA, BWF, and E were subjected to two successive drought cycles. With a drought-sensitive wheat cultivar, BWF shifted from 12 to 34%, negative OA occurred, and the RWC at turgor loss remained at approx. 86%. With a more resistant wheat cv., OA occurred, BWF remained at 10%, and turgor loss occurred at 80%. With a resistant wild wheat relative, BWF shifted down from 18%, yet the RWC at turgor loss shifted from 89% to 81%. In no case, did substantial increase in E enhance cell water status under drought. It was concluded that OA can afford substantial acclimation to droughted leaves, and that shift in BWF does not necessarily afford acclimation. Supported by NSF grant DMB 8706240.

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TURGOR-REGULATED SUGAR RELEASE IN SUGARBEET LEAVES

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Under drought conditions, sucrose distribution (osmotic adjustment vs. export) may be regulated at the mesophyll plasmalemma/tonoplast. Leaves were given $^{14}CO_2$ for a 30/30 min pulse/chase. Peeled leaf discs were put in low or high osmotic solutions to monitor release of labeled solutes. High turgor increased efflux rates double those at low turgor. About 30% and 55% of the released label was in the sugar (sucrose + hexose) fractions at low and high turgor, respectively. Response to changes in cell turgor was rapid and reversible. PCMBS had no effect on efflux. NEM and CCCP enhanced efflux at high turgor. Presence of unlabeled sucrose in the wash solutions greatly enhanced sucrose efflux in a turgor-dependent manner; suggesting the presence of a sucrose exchange system, which appeared to be at the tonoplast. Turgor-regulated efflux involved both the tonoplast and plasmalemma. However, efflux across the plasmalemma was not carrier-mediated. NSF funded (DCB-87-18303).

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ASSOCIATION OF IN SITU STROMAL VOLUME CHANGES WITH WATER STRESS INHIBITION OF PHOTOSYNTHESIS IN SPINACH Santakumari Mane & Gerald Berkowitz Hort. Dept., Rutgers Univ., New Brunswick, NJ 08903

Previous research has indicated that in water-stressed plants, inhibition of photosynthesis (PS) may be associated with protoplast and/or chloroplast volume changes, and therefore, that cellular-level acclimation to stress may result from volume maintenance at low water potential (Ψw). To determine which factor is most closely associated with changes in PS during water stress in situ chloroplast and protoplast volumes, and PS, were monitored in spinach exposed to two successive water stress cycles. Results indicated that the chloroplast can undergo osmotic adjustment independently, and above that experienced by the cell as a whole. The extent of stromal volume maintenance during the second stress cycle (as compared to the first) far exceeded the extent of protoplast volume maintenance, and correlated well with acclimation of PS to low Ψw. Leaf osmotic adjustment capability was no greater during the second cycle than the first, but stromal volume at low Ψw was greater during the second cycle. Supported by NSF grant DMB 8706240

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EFFECT OF WATER STRESS ON CHLOROPHYLL AND CAROTENOID CONTENTS ON SEEDLINGS FROM THREE SEED SOURCES OF PINUS PONDEROSA.

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The effect of water stress on pigmentation was studied on seedlings from three seed sources of ponderosa pine (Pinus ponderosa Dougl.) selected from similar latitudes but along a gradient of decreasing water availability from the California coast to the western and eastern sides of the Sierra Nevada Mountains. All plants were grown in a common garden for 18 months. Water was withheld from the two-year old potted seedlings and weekly needle samples were taken for the 10 week drought period and for a two week recovery period. Pigments were passively extracted in dimethylformamide and quantified spectrophotometrically. The seedlings from the more mesic seed zone exhibited water stress earlier (Week 8) and to a greater extent (-1.53 Mpa predawn water potential) than seedlings from the other two seed zones. However, chlorophyll and carotenoid concentrations did not correspond to increasing level of water stress. There were differences in pigmentation among the seedlings from the three seed zones, with those seedlings from the west side of the Sierra Nevada having less chlorophyll a and b, and carotenoids than those from the other two regions. Analysis of the chlorophyll a/b ratio indicated that although there were differences in pigmentation, the relative abundance of chlorophyll a to b was consistent among seedlings from all three seed zones.

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MECHANISMS OF STOMATAL SENSITIVITY TO CHANGES IN LEAF WATER POTENTIAL IN PHASEOLUS ACUTIFOLIUS

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Phaseolus acutifolius (Pa) tolerates drought by postponing dehydration by a deeply penetrating root system and sensitive stomata that close at higher leaf water potentials (LWP) than the drought sensitive P. vulgaris (Pv). Little is known, however, about the structural or functional differences that contribute to the differential response to LWP. Using a continuous flow porometer, we compared the kinetics of stomatal opening and closing in response to rapid changes in LWP. Leaf excision resulted in greater and more rapid transient increase in stomatal conductance in Pa than Pv. These results suggest that the sensitivity of Pa stomata is more likely connected to turgor potentials of the stomata/subsidiary cell complex and less likely related to chemical signals.

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MATRIC POTENTIAL OF CELL WALL: QUESTION OF SENSING TURGOR REDUCTION AND SIGNAL TRANSDUCTION UNDER WATER STRESS

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Water deficit, a stress largely physical and associated usually only with a very slight reduction in water mole fraction, can effect profound alterations in metabolism. How water stress is sensed by the plant and transduced into metabolic changes remains unresolved. It is often thought that macromolecules located at the plasm membrane-cell wall interface (MWIF), as well as the membrane, could be distorted sufficiently by reductions in pressure difference across the MWIF, due to turgor reduction by water stress, to trigger changes in metabolic reactions. Theories of physics, however, suggest that matric potential of the cell wall water is really tension within the water. Hence, with water stress, the pressure differential across the MWIF may be maintained because tension in the wall would increase in step with the reduction in protoplasm turgor. Other means of turgor transduction are also considered.

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USE OF INTERSPECIFIC GRAFTS TO TEST THE CONTRIBUTION OF ROOT SYSTEMS TO DROUGHT TOLERANCE IN BEANS

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Phaseolus vulgaris L. (Pv) is drought sensitive compared to Phaseolus acutifolius Gray (Pa). Pa postpones dehydration: it maintains a higher water potential and a higher water use efficiency and has more sensitive stomata than Pv. We would like to know whether this is determined by the roots or the shoot. To answer this question, we studied the root system's effect on plant water relations using interspecific reciprocal grafts. When plants were well-watered, the shoot determined leaf growth, leaf water potential and photosynthesis. Root system's effects on growth, water relations and photosynthesis under water stress will also be presented.

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NON-HYDRAULIC SIGNALS FROM ROOTS IN DRYING SOIL: INHIBITION OF LEAF ELONGATION BUT NOT STOMATAL CONDUCTANCE

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The effects of soil drying on leaf elongation rate and stomatal conductance were examined in maize (Zea mays L.) plants having roots split between two soil containers. One container was allowed to dry while the other was maintained at saturation. Rapid soil drying resulted in a 35% maximum inhibition of leaf elongation. Stomatal conductance was not affected. The inhibition of leaf elongation was not caused by reductions in shoot water or nutrient status. Maximum inhibition occurred during the light hours and the effect was observed only when the soil water potential declined to below that of the leaves. Midday root water potentials (-0.29 MPa) were much higher than that of surrounding soil (-1.0 MPa), however. The results suggest that signal transport requires rehydration of roots in dry soil during the dark period. The soil drying treatment had no effect on stomatal conductance despite variations in light, humidity and plant nutrition.

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AMMONIA ASSIMILATION DURING THE MICROPROPAGATION OF VITRIFIED Agave fourcroydes.

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In vitro propagation of plants is frequently associated with a disorder named vitrification. The main characteristics of this phenomenon are the relative absence of cellular organization and vascular tissue. Among other factors, the water potential of the medium and the nitrate-ammonium ratio could be the elicitors of this phenomenon. Therefore the aim of this work is to determine if there is any relationship between vitrification, the water potential of the medium and the nitrogen assimilation enzymes in Agave fourcroydes. Though the multiplication of new shoots present vitrification symptoms at low water potentials (-3.5 bars), they disappear at higher potentials. The enzymes' levels at this stage suggest that GS/GOGAT is the main pathway of ammonia assimilation, even in the vitrified plantlets. However GDH could be important at low water potentials.

Partially supported by CONACYT, Grant No. PCECCNA-050789.

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NITROGEN AND WATER STRESS, AND ACCLIMATION IN LODGEPOLE PINE

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We determined the effects of interacting nitrogen (N) and water stresses on lodgepole pine (Pinus contorta var. latifolia) seedling growth and physiology, and tested acclimation to these stresses. First-year seedlings were grown in solution culture with fixed exponential N addition rates (Ra) and polyethylene glycol induced water stress. The only interaction was the antagonistic effect of water stress on RGR response to N. Foliar N concentration increased and N use efficiency decreased with increasing N Ra. Root:shoot ratio was inversely related to N Ra, but was lowest in moderate water stress conditions (-0.5 MPa); effects of preconditioning N and water stress were maintained through subsequent treatments. Shoot water potential and stomatal conductance were unaffected by N, and lowered by water stress, though preconditioning moderated the latter response. The effectiveness of N fertilization is reduced at high application rates and by water stress.

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PROLINE ACCUMULATION AND FERREDOXIN ISOPROTEIN VARIATION OF Mesembryanthemum crystallinum AS RESPONSES OF SALT STRESS

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A facultative halophyte, M. crystallinum transfers metabolism from C_3 mode to CAM mode under the salt stress. Rapid accumulation of proline is observed at the early stage of CAM transition. We found that this plant had two Fd isoproteins (FdI and FdII) and the ratio of FdI/FdII varied with the transition to CAM. In the C_3 mode plants the ratio of FdI/FdII was about 1.6 and those of CAM mode plants increased up to 10. When salt stress (0.4 M NaCl) was given to the 8 week old C_3 mode plants, the rapid increase of the Pro level and the subsequent variation of Fd isoprotein ratio were observed. On the other hand, the CAM mode plants transferred into the low salt conditions were released from the Pro accumulation. However, Fd isoprotein ratio did not revert easily.

We discuss about the relationships among Pro accumulation, Fd isoprotein variation and CAM transition.

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COMPARATIVE BIOCHEMISTRY OF BETAINE BIOSYNTHESIS AND ACCUMULATION IN DIVERSE DICOT FAMILIES.

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Salt stress elicits betaine accumulation to high levels in species from several diverse dicot families (Chenopodiaceae, Amaranthaceae, Convolvulaceae, Solanaceae, and Asteraceae). FAB-MS studies with deuterated precursors showed that species from all these families synthesize betaine from choline. Enzyme assays and immunotitration data showed that all accumulating species contained betaine aldehyde dehydrogenase (BADH) enzyme activity recognized by antibodies raised against purified BADH isolated from Spinacia oleracea. Immunoblotting indicated that the BADH monomer was in all cases of Mr ≈63,000. The similarity of BADH in the different species is consistent with a single evolutionary origin for the betaine pathway. This was supported by the presence in immunoblots of a cross-reacting band at Mr ≈63,000 in Magnolia x Soulangiana, a primitive angiosperm.

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BETAINE SYNTHESIS AND ACCUMULATION IN ALLIGATOR WEED CELL SUSPENSION CULTURE

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Alligator weed (Alternanthera philoxeroides) cell suspension cultures were established in a Murashige-Skoog media with 1 mg/mL 2,4 D (MS) to study betaine synthesis and betaine uptake. Cell suspensions were subcultured to: MS media (control), MS + 5 mM betaine, a salt-shock treatment (MS + 200 mM NaCl) and a salt-shock plus betaine treatment (MS + 200 mM NaCl + 5 mM betaine). After 21 days, growth in salt-shock cultures was reduced 75% and growth in salt-shock plus betaine was reduced 50%, as compared to control cultures. Relative cell betaine concentrations were: salt shock plus betaine > MS + betaine > salt shock > control. Betaine concentration in salt shock was 8X and in salt shock plus betaine 28X that in the control. Concomitant with the ability of these cultures to withstand a 200 mM increase in NaCl are the the abilities to produce betaine and to take up exogenous betaine.

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PROLINE DEPOSITION INCREASES IN THE GROWING ZONE OF MAIZE ROOTS AT LOW WATER POTENTIALS

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Longitudinal expansion of maize (Zea mays cv WF9xMo17) primary roots is unaffected by vermiculite water potentials as low as -1.6 MPa in the apical part of the growth zone, but is inhibited progressively with increasing distance from the apex (Plant Physiol. 87:50-57). Measurement of proline content and osmotic potential in serial 1-mm segments showed that about 50% of the osmotic adjustment in the growing apical region is accounted for by increases in proline concentration (up to 120 µmol/g H_2O). Use of growth kinematics showed that the net rate of proline deposition (µmol/mm length/hr) in the apical 4 mm of roots growing at -1.6 MPa increases by up to 7-fold. In contrast, increases in the concentrations of other solutes studied (K^+, sucrose, hexose) are greater in the vacuolated tissue farther from the apex, and can be accounted for by less solute dilution due to slower tissue volume expansion.

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PROTEIN METABOLISM IN ANAEROBICALLY-GERMINATED SEEDLINGS OF *ECHINOCHLOA PHYLLOPOGON*

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Although most plants cannot grow under flooded conditions, *E. phyllopogon* germinates and grows for extended periods without O_2. Our goal was to characterize the adaptations that permit survival and growth in low oxygen habitats. We have examined protein profiles of *E. phyllopogon* grown under aerobic and anaerobic conditions. When seedlings grown in air for 5 days were transferred to N_2, the polypeptide profile changed. Polypeptides of 22, 23, 42, 55, and 62 kd were enhanced by up to 3 days of anoxia. To determine if these changes were tissue specific, seedlings were divided into shoot, root, and seed, and the polypeptide profiles of each fraction were analyzed. The pattern of protein induction was similar between shoots and whole seedlings. For seed tissue, only minor polypeptide differences were observed when seedlings were transferred from aerobic to anaerobic conditions. Conversely, when seedlings grown in N_2 for 5 days were transferred to air, new polypeptides of 26, 44, 45, and 60 kd were observed in shoot tissue. These results suggest that increased glycolysis is one response of *E. phyllopogon* to low oxygen stress. Additional research is being conducted to determine other specific responses to flooding.

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INDUCTION OF NITRATE TRANSPORT IN MAIZE ROOTS, AND KINETICS OF INFLUX, MEASURED WITH NITROGEN-13

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Unlike phosphate or potassium transport, uptake of nitrate by roots is induced, in part, by contact with the substrate ion. Plasmalemma influx of ^{13}N-labelled nitrate in maize roots was studied in relation to induction of the uptake system, and the influence of short-term N-starvation. Roots not previously exposed to nitrate had a constitutive transport system, but influx increased 250% during 6h contact with 100μM nitrate, by which time the transport mechanism appeared to be fully synthesized. A 3d period of N-starvation prior to measurement of nitrate influx induced a greater capacity to transport nitrate than in unstarved controls, but this was only wholly expressed if roots were kept in contact with nitrate for the 6h needed for full induction. A kinetic analysis indicated a 160% increase in Vmax with insignificant change in Km. Full expression of the nitrate-contact induced system was dependent upon mRNA synthesis. An inhibitor of nuclear-coded protein synthesis eliminated the formation of the transport system while inhibition of mitochondrial-coded protein synthesis had no effect. Poisoning of membrane-bound proteins effectively disabled both the constitutive and induced transport systems.

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PHYSIOLOGICAL INDICATORS OF NITROGEN RESPONSE IN SHORT-ROTATION SYCAMORE PLANTATIONS

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American sycamore (*Platanus occidentalis* L.) seedlings were grown in the field under urea-nitrogen fertilization regimes to identify physiological variables that characterize the growth responses. Treatments included trees fertilized at the beginning of the growing season with 450 kg N/ha, trees fertilized periodically (three times during the growing season) at 37.5 kg N/ha, and unfertilized controls. Aboveground biomass accumulation in the heaviest nitrogen treatment was three times that of the controls, and nearly as much growth occurred when less nitrogen was added periodically. Photosynthesis, chlorophyll concentrations, and growth increased rapidly after a midseason application of a small amount of nitrogen, but not to a late-season application. There was no evidence that fertilization extended the physiologically active season or increased susceptibility to drought or cold. Sycamore leaves accumulated sucrose and mannose in response to water and cold stress in all treatments. Photosynthetic pigment concentrations, net photosynthetic rate, and leaf nitrate reductase activity were sensitive indicators of nitrogen fertilization, but foliar concentrations of nitrate, total nitrogen, soluble carbohydrate and soluble protein were not.

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EFFECTS OF ACIFLUORFEN ON CHLOROPHYLL INTERMEDIATES

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Photobleaching herbicides like acifluorfen (AF) cause accumulation of protoporphyrin IX (PPIX) due to inhibition of protoporphyrinogen IX oxidase (Matringe et al.), resulting in deregulation of the porphyrin pathway. Chlorophyll intermediates other than PPIX have been reported to accumulate in response to these herbicides. We examined the effects of AF and chlorophyll synthesis modulators on the accumulation of PPIX, Mg-PPIX, Mg-PPIX monomethylester (ME), and protochlorophyll (PChl) in cucumber tissues. In etiolated tissues, only PPIX accumulated above control levels in AF-treatments. In δ-aminolevulinic acid- (ALA) treated tissues, PPIX, Mg-PPIX, and Mg-PPIXME all accumulated above control levels, however, PPIX levels were only about 10 % of levels of AF treatments. There was no difference between the profiles of treatments with AF alone or AF plus ALA. ALA and dipyridyl (DP) increased PPIX levels caused by AF in green tissues. AF prevented or reduced ALA- or DP-stimulated Mg-PPIX, Mg-PPIXME, and PChl levels in green tissues. These data indicate that PPIX is the only chlorophyll intermediate caused to accumulate in AF-treated tissues, and that AF blocks carbon flow to intermediates after PPIX.

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THE INDUCTION OF PROTEINASES IN CORN AND SOYBEAN BY ANOXIA

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This study characterized the anaerobic changes in proteinase activities in corn and soybean roots and to investigate the possibility that these changes might contribute to the differential anaerobiosis tolerance of the two species. After 24 h of anoxia, crude protein extracts from H60 corn and Keller soybean root tips (10cm) were assayed for proteinase activities at pH range from 4.5 to 9.5. Turnover of aberrant proteins was studied in seedlings labelled with ^3H-leucine for 12 h under: a) puromycin (0.64 mM) in air, b) ethanol (1%) in air, c) nitrogen and d) air. After the treatment, the labelled proteins remaining in roots were determined every 2 h for 6 h. In both corn and soybean, activities of alkali proteinases increased, and activities of acid proteinases declined under anoxia. Neutral proteinases increase in anoxic corn roots, but decline in anoxic soybean roots. The protein turnover rate in corn treated with puromycin, ethanol and nitrogen was much higher than in control roots. The protein turnover rate in soybean roots treated with puromycin, ethanol was similar to the rate of the control. The results indicated that: a) anoxic corn can degrade aberrant proteins, but anoxic soybean cannot, b) the degradation of aberrant proteins in anoxic corn is accomplished by neutral proteinases, and c) the accumulation of aberrant proteins in soybean might contribute to the susceptibility of this species to anoxia.

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EFFECTS OF ANAEROBIOSIS ON IN VIVO PROTEIN SYNTHESIS IN THE ROOTS OF A MARINE ANGIOSPERM ZOSTERA MARINA L.

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The roots of the temperate seagrass Zostera marina undergo daily periods of anaerobiosis at night. These diurnal periods of anoxia alter many metabolic processes in the roots including carbon and nitrogen metabolism, amino acid synthesis, and synthesis and levels of ATP, ADP and AMP. To further characterize the effects of anaerobiosis, we determined in vivo rates of protein synthesis by measuring the relative incorporation of 35S-MET in TCA precipitated protein samples. Results from these studies show that in vivo protein synthesis decreases continuously during 12 h of anaerobiosis and correlates with changes in ATP levels under similar conditions. Furthermore, polypeptide patterns obtained by SDS-PAGE and 2D-SDSPAGE indicate that anaerobiosis leads to differential protein synthesis in the roots.

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ANAEROBIOSIS AND ETHANOL EFFECTS ON GERMINATION, GROWTH, AND PROTEIN SYNTHESIS OF FIVE *ECHINOCHLOA* SPECIES

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Five *Echinochloa* species, encompassing a spectrum from flood tolerant to flood intolerant, were studied to determine the mechanisms of anaerobic germination and growth. Seeds were germinated in air or N_2, plus 0, 1 or 3% ethanol, and germination rates and growth measurements recorded for 7 days. In air or N_2, increasing ethanol levels did not affect total germination *per se*, although the rate of germination was delayed in N_2. Shoot/root lengths in air were highest for tolerant species and increased with increasing ethanol, whereas, in intolerant species, shoot/root lengths decreased with increasing ethanol. Aerobic *vs.* anaerobic polypeptide profiles of each of the species were compared by SDS/PAGE. For all species, the number of polypeptides decreased under anaerobiosis and several quantitative differences were apparent relative to the aerobic profile. In addition, amino acid incorporation into protein was analyzed by [^{35}S]-Met labeling of 3 day old seedlings grown in air or N_2. Significant protein synthesis was measured in tolerant seedlings under N_2 and several polypeptides were specifically induced. These results are being compared with labeling patterns of the other semi-tolerant and intolerant *Echinochloa* species to determine their importance in flooding tolerance.

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RATES OF GLYCOLYSIS IN AEROBIC AND ANAEROBIC MAIZE ROOT TIPS

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Hypoxically pretreated HPT, (4% O_2, 25C, 16h) and non-hypoxically pretreated NHPT, (40% O_2, 25C, 16h) maize root tips were examined for the presence of a 'Pasteur effect' using respirometry. The respiratory quotient under aerobic conditions with 50 mM glucose was 1.0, indicating glucose was being utilized as the predominant carbon source. The amount of glucose undergoing glycolysis under aerobic and anaerobic conditions could then be calculated. Glycolysis was accelerated under strictly anaerobic conditions (anoxia) only in root tips that were HPT. There was then nearly a two fold increase in glycolysis, compared with HPT tissue that was returned to fully aerobic conditions. NHPT tissue under anoxia continued to utilize glucose initially at the same rate as under 40% O_2. Output of CO_2 under anoxia was also examined in *Adh1* null root tips, which were found to behave similarly to wild type in production of CO_2 under aerobic and anaerobic conditions. Anaerobic ethanol production was measured in HPT wild type and *Adh1* null, and NHPT wild type and *Adh1* null root tips. HPT tissues consistently produced more ethanol regardless of the presence of ADH1. However, within the HPT class, wild type tissues produced more ethanol than did *Adh1* null root tips. These data suggest that a period of low oxygen partial pressure is necessary to permit acclimation of the root tip to anoxia. Further, ADH1 may not be essential for the acclimation process.

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INHIBITION OF PHOTOSYNTHESIS BY ROOT OXYGEN DEFICIENCY IN CUCUMBER (CUCUMIS SATIVUS): RELATION TO NUTRIENT ION CONCENTRATIONS IN LEAVES

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Over-watering the rooting medium (flooding) frequently inhibits growth of flood-sensitive species by interfering with the supply of oxygen to the roots. We have studied this condition in plants grown hydroponically, in a controlled environment room, with the roots temporarily made hypoxic by sparging with oxygen-free nitrogen gas. Root hypoxia induced stomatal closure and inhibited net CO_2 fixation. After 7 days of hypoxia, stomatal conductance was only 9-18% of controls, even at high relative humidity (80-90% RH). Net CO_2 fixation rate of treated plants declined to 15% of the controls at ambient concentrations of CO_2. When net CO_2 fixation was measured as a function of substomatal CO_2 concentration, the maximum carbon exchange rate at saturating concentrations of CO_2 was 42% of the controls after 5 days of hypoxia. Thus, slowing of CO_2 fixation could be attributed to inhibition at the chloroplast level. The relation between changes in leaf gas exchange, and inhibition by hypoxia of ion transport from roots to leaves will be described.

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GROWTH AND PHYSIOLOGICAL CHANGES OF SHORTLEAF PINE SEEDLINGS EXPOSED TO ACID RAIN AND OZONE

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Four genotypes of shortleaf pine (Pinus echinata Mill.) were grown in open-top chambers in the Piedmont region, S.C. The seedlings were exposed to a combination of ozone and acid rain treatments. Baseline data were obtained for seedling heights, diameter of stems, carbohydrate, chlorophyll and protein content of pine needles. Similar measurements were made after 4 and 8 weeks of exposure. Height data showed reduced growth for low pH and high ozone treatments, especially in combination. Reducing sugar content dropped by 20% after 4 weeks but returned to baseline levels after 8 weeks of treatment. Starch levels showed an inverse relationship to reducing sugar levels. Changes in chlorophyll and protein content are also discussed.

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EFFECTS OF CLOUDS AND OZONE ON RED SPRUCE SEEDLINGS

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Potted native and Phyton-grown (Phyton Technologies) red spruce seedlings were placed in open-top field chambers constructed on Whitetop Mountain, VA (elevation 1680 m) to evaluate the effect of ozone and acid cloud deposition on seedling growth and metabolism. Chamber treatments were (1) exclusion of clouds and an approximate 50% reduction in ambient ozone, (2) ambient ozone with clouds excluded, and (3) exposure to clouds and ambient ozone (control). No differences were detected between chamber treatments for diameter growth, total chlorophyll, chl a and b, chl a/b ratio, and carotenoids. No enhancement of photosynthesis and respiration was seen in exclusion chambers for current and previous year's growth of native seedlings during the growing season. Photosynthesis of Phyton-grown seedlings was consistently higher in exclusion chambers compared to control chambers over the course of the growing season, although differences were not statistically significant. After one growing season, neither pollutant had significant effects on seedling growth and metabolism.

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PHYLOGENETIC DISTRIBUTION OF POLYPHENOL OXIDASE AND ITS PHYSIOLOGICAL IMPLICATIONS

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The physiological function of polyphenol oxidase (PPO) may involve the dissipation of toxic oxygen species. If so, the presence of PPO could be expected to correlate phylogenetically with the evolution of terrestial plants. The presence of PPO was tested in green algae (aquatic and lichen symbionts), mosses, hornworts, and liverworts by cytochemical, electrophoretic, and spectrophotometric methods. All green algae (Chlorella, Stigeoclonium, Microspora, and Spirogyra) tested had no detectable PPO except a lichen symbiont (a Trebouxioid alga). The moss Dicranium had no detectable PPO, however, the liverwort Conocephalum and the hornwort Phaeoceros had easily detectable PPO activities. Marsilea, a fern, and Selaginella, a fern ally, also had PPO activity. The M_r as detected by electrophoretic mobility of PPO varied from 36 to 45 kD, depending on the species. These data are consistent with the view that PPO could function in dissipation of excess molecular oxygen in chloroplasts of terrestial plants.

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EFFECTS OF IN VITRO OZONE TREATMENT ON PROTEOLYSIS OF PURIFIED RUBISCO FROM TWO HYBRID POPLAR CLONES

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Plants exposed to ozone (O_3) exhibited symptoms of premature senescence, including early decline in quantity of rubisco. O_3-induced oxidation may cause changes in protein conformation of rubisco, resulting in enhanced proteolysis. To test this hypothesis, rubisco was purified from two hybrid clones of Populus maximowizii x trichocarpa, clones 388 and 245, and treated in vitro with O_3 or air. Rubisco was then challenged with bromelain, papain, chymotrypsin, carboxypeptidase A, or endoproteinase Glu-C and percent degradation measured by SDS-PAGE and densitometric scanning of the gels. Degree of rubisco sensitivity to oxidation may be related to available sulfhydryl (SH) groups on the protein. The number of SH groups in native and denatured rubisco was measured for purified rubisco of both clones by DTNB titration method. The relationship between sensitivity to proteolysis and number and availability of SH groups will be discussed.

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RESPONSE OF PHOTOSYNTHESIS AND THE CELLULAR ANTIOXIDANT SYSTEM TO OZONE IN POPULUS LEAVES.

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Atmospheric ozone causes various highly reactive intermediates (eg. peroxyl and superoxide radicals, H_2O_2, etc.) to form in plant tissues. A plants productivity in environments with ozone may be related to its ability to scavenge the free radicals formed. The effects of ozone on photosynthesis (PS) and some free radical scavengers were measured in poplars (fifth emergent leaf). Clonal poplars (P. deltoides X P. cv. caudina) were grown and fumigated with 180 ppb ozone for 3 h at appropriate times. Total glutathione (GSH & GSSG) increased in fumigated leaves. The ratio of GSH/GSSG decreased from 12.8 to 1.2 in fumigated plants. Superoxide dismutase levels increased 2-fold in fumigated plants. PS declined approx. 50% in fumigated plants even though electron transport and NADPH levels were unaffected. We speculate that reduced PS may be due to a diversion of energy to ozone adaptation processes.

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UV-INDUCED SYNTHESIS OF HYDROGEN PEROXIDE

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Suspension-cultured rose cells irradiated with UV (254 nm, 558 J m^{-2}) showed a transient efflux of K^+, and a production of H_2O_2 measured by chemiluminescence of luminol in the presence of peroxidase. The peak concentration of H_2O_2, attained at about 60-90 min after irradiation, was 2-5 uM. The addition of superoxide dismutase to irradiated cells stimulated luminescence, suggesting that the H_2O_2 came at least in part from superoxide that was present in the extracellular medium. Treatments that inhibited the UV-induced efflux of K^+ also inhibited the appearance of H_2O_2, though the converse was not always true, suggesting that K^+ efflux was necessary for H_2O_2 synthesis, but not vice-versa. H_2O_2 in the extracellular space is required for lignin synthesis in many plant tissues. Phenolic compounds, the other substrates for lignin, are induced by UV. We suggest that the UV-stimulated production of H_2O_2 is part of a coordinated induction of lignin synthesis.

Supported in part by EPA Grant R-813229-01-1.

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OZONE "STRESS PROTEINS" IN PEA PLANTS

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21 day old pea plants were fumigated with 200, 100, 50 and 0 ppb ozone (8 hrs/day) for 5 days. Soluble proteins were extracted from the first 6 leaves and analysed by 1D SDS PAGE. Polypeptides were visualised after coomassie blue staining. With respect to controls, fumigation resulted in a dose dependent decrease in staining intensity of several polypeptides (of approximate M.W. 94, 54, 35 kD). However, treatment with 200 ppb ozone resulted in the appearance of a polypeptide with a molecular weight of circa 32 kD. This polypeptide was absent from control (0 ppb ozone) plants. Currently, we are (1) purifying the c32kD polypeptide, (2) studying the temporal aspects of the synthesis of this polypeptide and (3) investigating whether this represents a general response to pollutant gasses.

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ASSESSING OZONE INJURY TO LEAVES OF PHASEOLUS VULGARIS (PINTO) BY SURFACE TEMPERATURE IMAGING

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Ozone injury to leaves can be assessed several ways, but most involve waiting for the injury to become visible or using destructive chemical methods. Also most methods can not discriminate regions of differential injury. An infra-red thermal imaging system can be used to monitor surface temperature changes, partially mirroring changes in transpiration rate induced by ozone injury. Plants were exposed to various levels of ozone and analyzed by the infra-red image patterns pre- and post-fumigation. Leaves (2 and 24 hr post-fumigation) showed an increased surface temperature even under conditions where visible injury was not apparent. The increase in surface temperature correlated with reductions in transpiration. Under carefully controlled irradiation conditions the infra-red image can be used as a non-destructive method to assess ozone injury.

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CHANGES IN mRNA AND PROTEIN CONTENT OF SO_2-FUMIGATED MAPLE LEAVES

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The effect of acute SO_2 fumigation on foliar DNA, RNA, and protein levels in 2-yr-old containerized Acer seedlings was examined. While DNA content did not change appreciably in either SO_2-sensitive red maple (A. rubrum L.) or SO_2-tolerant silver maple (A. saccharinum L.), significant reductions in mRNA (35% for red maple; 21% for silver maple) were observed after 54 h fumigation (6 h/day x 3 days/wk x 3 wk) at 2.5 ppm SO_2. Reductions in mRNA and protein content were accompanied by a corresponding decline in net photosynthesis (Pn). The data from this study suggest that acute SO_2 fumigation alters Pn in red and silver maple by disrupting molecular events, and that species sensitivity for these particular Acer spp may be related to the degree of change associated with mRNA and total protein content.

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PATTERNS OF STABLE CARBON ISOTOPE DISCRIMINATION ($\delta^{13}C$) IN PINUS TAEDA AS A FUNCTION OF OZONE STRESS

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This research tested the hypothesis that ozone stress shifts the isotopic composition of carbon ($\delta^{13}C$) in needles of Pinus taeda L. (loblolly pine) seedlings grown under realistic field conditions using open top chambers. Three different ozone exposure regimes were maintained for two growing seasons. At the end of the second year, foliage exhibited two statistically significant patterns in $\delta^{13}C$ values: (1) a decrease (more negative) from -24.86 to -28.16 per mil with needle age and (2) an increase from -27.15 to -25.95 per mil with increasing ozone stress. Whereas all needle age classes exhibited the same response as a function of ozone stress, the most pronounced shift in $\delta^{13}C$ was in the youngest age class. In conjunction with intensive gas-exchange studies, the direction and magnitude of the shift in response to ozone indicates that the pollutant's effects on foliar gas-exchange processes are greater on stomatal physiology than on carbon dioxide assimilation. Consequently, it is proposed that one of the seasonally integrated effects of elevated levels of ozone stress on foliage of P. taeda is lower stomatal conductance to water vapor and thus more efficient water use. An untested corollary is that seedlings grown at ambient levels of ozone stress are more susceptible to drought than their counterparts grown at elevated levels of ozono.

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OVERLOOKED RHIZOSPHERE STRESS FACTORS: SIMPLE VOLATILE COMPOUNDS RELEASED BY DECOMPOSING PLANT RESIDUES

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The decomposing residues of winter cover legumes, weeds, and crops, both dicots and monocots, released mixtures of volatile substances into the soil atmosphere. The simple organic constituents of the mixtures, singly and in combination, inhibited seed germination, radicle elongation, and early seedling development in a number of species. Exposure of cotton (Gossypium) to plant residue volatiles during seedling establishment resulted in whole plant "environmental stress" effects such as reduced vegetative biomass accumulation, leaf and flower abortion, and accelerated reproduction with significantly reduced yield. Individual components of vapor-phase mixtures released by a variety of plant residues have been identified using GC-MS; and comparative structure-activity relationships determined. The inhibitory activities of plant residue emissions were found to depend on the type and position of functional group substitution, degree of unsaturation of the carbon chain, molecular weight, volatility, and water solubility. The most inhibitory classes of compounds were C_4-C_9 alkenals, alkanals, 2-alkanones, and 2-alkanols. Individual compounds were inhibitory at micromolar concentrations, and the activities were additive.

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THE FUNDAMENTAL METABOLIC LESION IN PLANT STRESS: ALTERED CELLULAR COMPARTMENTATION AND INTERORGANELLE TRANSPORT

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In cotton (Gossypium spp.) a single environmental stress factor, soil temperature, can influence biomass accumulation, root:shoot ratio, plant height, leaf expansion, shape and color, root extension, branching, thickness, color and shape, flower production and fruit yield. These growth parameters are equally sensitive to nutrient and water deficits, air temperature, salinity, and flooding; a wide variety of stress factors that elicit strikingly similar responses in many plant cells and tissues. Such pleomorphic responses to differing stress factor combinations indicate that stress, like resource limitation, influences the basic metabolic processes of carbon fixation, respiration, transpiration, and water and mineral nutrient uptake and transport. Thus, the search for the sensory and regulatory mechanisms which allow a plant to maintain a functional metabolic homeostasis while adapting to a constantly changing environment should begin at the subcellular level where the essential balances between cellular processes and organellar activities are mediated by the various membranes responsible for maintaining intracellular compartmentation and interorganellar transport of metabolites.

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NITROGEN METABOLISM DURING SALT STRESS IN Catharanthus roseus CELL SUSPENSION CULTURES.

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Catharanthus roseus cell suspension cultures under low salt stress (0.17 M NaCl) lost 50% of their GDH activity; whereas GS showed a 2-fold activity respect to control cells. When salt stress was increased to 0.51 M NaCl, growth, respiration, viability and chlorophyll content decreased, while the amino acid pool increased as well as proline. GDH activity increased 2-fold but GS did not change. These data suggest that under high salt stress both pathways of ammonia assimilation are functional.

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WATER RELATIONS OF PRIMED TOMATO SEED GERMINATION

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Germinating primed seeds did not display a marked plateau in water uptake during imbibition. Both seed and embryo water contents were higher than those of non-primed seeds. However, embryo water and osmotic potentials were lower than those of embryos from non-primed seeds. The force necessary to puncture the endosperm and seed coat tissues opposing the radicle tip of primed seeds was lower than that of non-primed seeds. The rates of expansion of embryos from primed seeds were greater than those from non-primed seeds when expanding in a range of external osmotic potential, but both primed and non-primed embryos ceased to expand when placed in a -0.8 MPa solution. Priming advanced the timing of radicle emergence by improving the rate of water uptake by the seeds; by eliminating the time necessary for the loosening of embryo cell walls and by permitting the completion of the first step of the endosperm weakening process.

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THE ENVIRONMENTAL CONTROL OF SEED GERMINATION IN FIELD PENNYCRESS (Thlaspi arvense L.)

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The effect of environmental conditions during storage and imbibition on germination in field pennycress was investigated. Freshly harvested seeds exhibited primary dormancy, which was rapidly lost following one month afterripening in a dry state. Nondormant seeds were positively photoblastic. Germination was promoted by red light and inhibited by far red light. Seedling emergence was inhibited in the shade of a wheat canopy. Germination was more sensitive to moisture stress than in wild oat or wheat. Imbibition at 6°C induced a deep secondary dormancy. In contrast to primary dormancy, cold-induced dormancy was not alleviated by red light, alternating temperatures, or 2 months dry storage. However, exogenous GA_3 or 24 weeks dry storage did lead to germination in cold-induced dormant seeds. Secondary dormancy was not observed in seeds that were preincubated at 21°C for 1 or 2 days prior to cold treatment. The response of seeds in controlled environments is related to the germination behavior in the field.

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REQUIREMENT FOR ENDOGENOUS ETHYLENE DURING SEED GERMINATION: HOW WIDESPREAD IS IT AMONG PLANTS?

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Using inhibitors of ethylene synthesis and action, several recent studies have reported that endogenous ethylene is essential for the germination of *Lactuca sativa* and *amaranthus caudatus* (Physiol Plant 63:49; 67:584; Plant Physiol 81:780,950; 89: in press). We studied the requirement for endogenous ethylene among seeds of a range of species in order to determine whether this was a common feature among plants. The following criteria were used for this purpose: (i) Inhibition of germination upon inhibition of ethylene action by 2,5-norbornadiene; (ii) Suppression of ethylene evolution and germination by an inhibitor of ethylene synthesis, 2-aminoethoxyvinyl glycine; (iii) Restoration of germination by co-application of exogenous ethylene and the above inhibitors; (iv) Coincidence between the time course of ethylene evolution and germination. Only in a small proportion of the tested species was the germination found to require endogenous ethylene.

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IN VITRO GERMINATION TEST SYSTEM FOR COTTON SEED RADICLES

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The first visible event of cotton seed germination is emergence of the radicle at approximately 12hr of imbibition at 30°C. To test our hypothesis that sufficient food is stored in the radicle to support this initial growth, we developed an in vitro germination system. Excised radicles were inserted apex down in a support screen sitting inside a plastic container lined with moistened filter paper. The container was placed in an incubator at 30°C. Elongated radicles were removed at 24hr and examined with TEM. Fine structural changes in cortical cells, namely depletion of stored lipid and protein reserves, organelle elaboration and initial cell divisions, were similar to those in radicles of germinating whole seeds. These data indicate that radicles can be removed from the chamber and analyzed directly at any time of imbibition. We conclude, therefore, that the in vitro test system offers the advantage of being a simple and direct approach for determining the precise time when specific biochemical, cytological and physiological events occur in radicles of imbibing seeds.

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ISOLATION OF ARABIDOPSIS MUTANTS WITH ALTERED SEED FATTY ACID COMPOSITION

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By direct screening of Arabidopsis seed fatty acid methyl esters, we have isolated mutants which are deficient in the elongation of 18:1 to 20:1 and the desaturation of 18:2 to 18:3. Both the elongation and the desaturation mutants, designated MB14 and BL1 respectively, have only 10% of the wild-type levels of 20:1 and 18:3 in their seeds. The intermediate levels of 20:1 and 18:3 in F1 seeds of crosses to the wild type indicate that the level of enzyme is regulating the amount of 20:1 and 18:3 in seeds. Consistent with this observation, the mutations were found to segregate 1:2:1 in F2 seeds. We have found that the 18:2 desaturase mutation is clearly expressed in root phosphatidylcholine.

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PURIFICATION AND CHARACTERIZATION OF A PROTEINASE FROM RESTING JACK PINE SEEDS.

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Proteinase activity was investigated in resting Pinus banksiana L. seeds. Pepstatin A-sensitive enzyme was purified using anion exchange, gel filtration and affinity chromatography. The activity was monitored by measuring 9% TCA-soluble hemoglobin degradation products using Folin reagent. Salt and heat stability, as well as pH optima and inhibitor sensitivity were determined using the purified enzyme. The preparation was most stable at under slightly acidic (pH 5) and high salt (0.5M) conditions, while optimal activity was near pH 3.5. Hemoglobin degradation products were analysed by gel filtration and urea/SDS PAGE. The potential role of this enzyme in germination will be discussed.

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PURIFICATION AND CHARACTERIZATION OF PROTEASES DEGRADING SOYBEAN PROTEINS

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During soybean seed germination a number of proteolytic activities are capable of degrading soybean seed proteins. Protease K1 which peaks by day 4 of germination, modifies the native Kunitz soybean trypsin inhibitor, Ti^{α}, resulting in a new polypeptide, $Ti^{\alpha}m$, which lacks five amino acid residues from the carboxyl terminal. Protease K1, exhibits optimum activity on Ti^{α} at pH 4.0 and requires the presence of a reducing agent (2-mercaptoethanol). We have purified this enzyme from soybean seeds (Glycine max (L.) Merill cv. Amsoy 71) to 90% homogeneity by ion exchange chromatography on DEAE- and CM-Trisacryl, and by gel filtration on S-200 Sephacryl column. Protease K1, has been examined for substrate specificity, as well as susceptibility to various proteolytic inhibitory reagents. Supported by NSF Grant DMB 8301202.

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THE ROLE OF ABSCISIC ACID IN HEAT STRESS INDUCED SECONDARY DORMANCY IN APPLE SEEDS

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Exposure of stratified apple (Malus domestica Borkh. cv. Golden Delicious) seeds to a temperature of 30C induces secondary dormancy. To determine if a rise in abscisic acid (ABA) content was associated with the loss in germination capacity, stratified seeds (3,6, or 9 weeks at 5C) were held at 30C for 0,3, or 6 days. Seeds were dissected into seed coat, cotyledons, and embryonic axes and analyzed for ABA content. Stratification at 5C either had no effect or increased ABA content in embryonic axes, cotyledons, and seed coats. Exposure to 30C after stratification either did not affect or decreased ABA content in embryonic axes and seed coats; in contrast, cotyledonary ABA was increased. Seed coats, cotyledons, and embryonic axes held for 3,6, or 9 weeks at 20C contained the same or higher levels of ABA in comparison with non-stratified seeds or seeds stratified at 5C. ABA changes were not correlated with germination during stratification or after exposure to 30C. These data suggest that changes in ABA are unrelated to changes in dormancy.

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FLUENCE- AND NITRATE-RESPONSES DURING INDUCTION OF SECONDARY DORMANCY IN SEEDS OF SISYMBRIUM OFFICINALE

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Seed germination in Sisymbrium officinale depends on the presence of Pfr and, upon induction of dormancy, increasingly on nitrate. Dose-response relationships of both factors were studied under dormancy inducing conditions. During the first 120h at 15°C the maximum of the fluence-response curves remained constant, but whole curves shifted along the x-axis to higher fluence values. After 120h maximal germination decreased. Nitrate-response curves were biphasic during the first 48h. The low-dose response gradually disappeared while the high-dose response remained constant up to 120h. Thereafter maximal germination declined and the response curve shifted further to higher values. We conclude that induction of dormancy coincides with a decrease in affinity of the tentative Pfr-receptor X, followed by a decline in the number of active receptors. A similar conclusion may be drawn for the nitrate-receptor. The occurrence of a biphasic nitrate response may be explained by a biphasic uptake pattern for nitrate.

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ALTERATIONS IN GENOMIC DNA FOLLOWING LONG-TERM SEED STORAGE.

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We are investigating changes in the integrity of DNA sequences from seeds stored under ideal conditions for prolonged periods. Nucleic acid was isolated from single soybean (Glycine max L.) seeds from the 1958 harvest of the cultivars 'Henry' and 'Ross'. A modified "CTAB" extraction protocol was used to isolate DNA suitable for most restriction endonucleases and in sufficient quantities for genomic blot analysis. Denaturing agarose gel electrophoresis revealed extensive single strand breaks in DNA from 30 yr. seed when compared to DNA from 1 yr. seed. In vitro assays for the number of DNA pol I primer sites in non-denatured DNA confirmed this observation and allowed a preliminary estimate of the extent of strand scissions. Differences in sequence deterioration of specific regions within the soy genome is currently under investigation. When restricted DNAs were separated under denaturing conditions, genomic blots showed that individual 30 yr. seed DNA fragments were highly degraded. Determinations of DNA integrity following imbibition will also be presented.

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THE ROLE OF LIPIDS IN SEED DETERIORATION

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Experiments were conducted to determine the relationship between lipid content and physical properties and seed longevity. The physical properties of the lipid were measured using DSC. In a survey of over 75 seed species, the lipid content and the temperature at which the lipid melted did not significantly correlate with the rate of seed aging ($P > 0.5$). The heat of fusion of oil extracted from seeds generally ranged from 80 to 110 J/g oil and was also not correlated with seed longevity. The energy associated with the lipid melt in vivo was between 30 and 105 J/g oil. These values were correlated with the rate of seed deterioration ($P < 0.001$). It is suggested that the in vivo measurement of the heat of fusion of seed oils indicates the environment of the lipid matrix. High energies of melting reflect a fluid lipid that is not bound to other macromolecules. Conditions which increase the lipid fluidity, like high moisture levels, will also increase the rate of deterioration of seeds. These results suggest that the condition of the lipid may be critical to the kinetics of the deteriorative reactions during seed aging.

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OLIGOSACCHARIDE CHAINS OF CATIONIC PEANUT PEROXIDASE

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Oligosaccharide chains of peroxidase have received little attention. Yet it appears that they may be important in enzyme stability and antigenic characteristics (BBRC 156 (1988) 500). The present study is largely intended to examine the number and length of the major cationic peanut peroxidase oligosaccharides. Chemical deglycosylation of the 40 kD Con-A nonbinding and the 37 kD binding fraction brought the peptide Mr to a common 31.5 kD value. Peptide proteolysis and oligosaccharide filtration revealed a total of 5 chains with different Mr. The two forms of this peroxidase differ in the number of chains and the composition of some chains. These differences are probably responsible for the degree of affinity for Con A.

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SOLUBLE AND BOUND APOPLASTIC ACTIVITY FOR PEROXIDASE β-D-GLUCOSIDASE, MALATE DEHYDROGENASE, AND NON-SPECIFIC ARYLESTERASE IN BARLEY AND OAT PRIMARY LEAVES

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Ca. 1% of the soluble protein, <0.3% of the glucose-6-phosphate dehydrogenase (G6P-D) activity, but up to 20% of the peroxidase (PER) and β-D-glucosidase (βDG) activity of barley or oat leaves was extracted by vacuum infiltrating peeled leaves with 200 mM NaCl pH 6.9 [centrifugation of intact leaves yielded more G6P-D but less soluble protein or PER activity]. Segments were homogenized and assayed for soluble cytoplasmic activity. Ionically-bound cell wall enzymes were solubilized in 1 M NaCl. The final pellet was assayed for covalently-bound activity. Activity in the soluble plus bound apoplastic fractions accounted for up to 76% of the βDG activity, 36% of the PER, 11% of the arylesterase, 4% of the MDH, but <2% of the G6P-D of peeled leaf segments.

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PURIFICATION AND CHARACTERIZATION OF β-AMYLASE FROM PEA EPICOTYLS

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β-Amylase was purified over 300-fold from etiolated pea epicotyls using DEAE-cellulose ion exchange and Sephadex G75 gel filtration column chromatography followed by glycogen precipitation and native gradient PAGE. The enzyme was shown to be a β-amylase by its ability to hydrolyze starches, its failure to hydrolyze starch azure, β-limit dextrin, and pullulan, and by the characteristic color of its end product complexed with KI/I_2 in starch gel transfer blots. The native and subunit mol wts were determined to be 55 and 57 kD, respectively, indicating that the enzyme is monomeric. The enzyme's pH optimum was 6.0 and the pI was 4.3. The Arrhenius E_a was 6.28 kcal/mole. Substrate specificity and effects of metal ions, sulfhydryl reagents and inhibitors will be discussed.

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A PARTIAL CHARACTERIZATION OF THE MAJOR ENDOAMYLASE IN PEA COTYLEDONS, LEAVES AND STEMS

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The major endoamylase from the cotyledons, leaves and stems of pea seedlings has been purified utilizing affinity chromatography (cycloheptaamylose coupled to Sepharose 6B). Purified amylases from all three organs comigrate during native PAGE with an endoamylase extracted from the apoplast of pea stems (Beers & Duke 1988 Plant Physiol 87:799-802) and are identical with respect to mol wt (43 kD, SDS) and pI (4.7), indicating that they are probably the same isozyme. Photosynthetic tissues yielded 1-2% of endoamylase activity found in cotyledons. The cotyledon amylase purified >1750 fold has a broad pH optimum of 5.5-6.5. It requires calcium and is not active in the presence of EDTA. Calcium protects the enzyme from heat inactivation. The cotyledon amylase hydrolyzes boiled Lintner starch, starch azure and β-limit dextrin. No activity was detected with β-glucan, PNP-glucose, nigeran, pullulan, and pea starch grains as substrates.

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PURIFICATION AND PROPERTIES OF β-XYLOSIDASE FROM CUCUMBER SEEDS

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β-xylosidase (E.C. 3.2.1.37 β-D-xyloside xylohydrolase) activity was detected in immature cucumber seeds. The enzyme had two molecular forms; a major form localized in the 30-60% $(NH_4)_2SO_4$ fraction and a minor form localized in the 60-95% fraction. When applied to SP-Sephadex C50 ion exchange resin at pH 5, the major form was unbound whereas the minor one was bound and eluted with 0.1 M NaCl. The major xylosidase was purified 25-fold after sequential chromatography on SP-Sephadex C50, Bio-Gel HTP hydroxyapatite, Sephadex G-200 and Concanavalin A-Sepharose 4B. The enzyme was a glycoprotein and had a native mol. wt. of 68.6 kD as determined by gel filtration. SDS-PAGE indicated a major band with a mol. wt. of 68 kD, although other protein bands of lower mol. wt. were still present. The major xylosidase exhibited optimal activity at pH 4 and 62.5°C. We are continuing to purify and characterize both molecular forms.

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EVOLUTIONARY, ENVIRONMENTAL AND TISSUE CONTROLS ON THE OCCURRENCE OF MULTIPLE ISOFORMS OF ACYL CARRIER PROTEIN

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Previous research has revealed that several higher plant species have multiple isoforms of acyl carrier protein (ACP). We have examined the development of this trait in evolutionarily diverse species. Isoforms were resolved by Western blotting and native PAGE of ^{3}H-palmitate labelled ACP's. Multiple isoforms of ACP were observed in primitive vascular plants including gymnosperms, ferns and Psilotum and the nonvascular liverworts and mosses. Therefore, the development of ACP isoforms occurred early in evolution. However, unicellular algae and bacteria such as Chlamydomonas, Dunaliella, Synechocystis and Agmnellum have only a single electrophoretic form of ACP. Thus, multiple forms of ACP do not occur in all photosynthetic organisms but may be associated with multicellular plants. We have also examined light and tissue control over the expression of ACP isoforms. The expression of multiple forms of ACP in leaf of Spinacia and Avena is altered very little by light. Rather, the different patterns of ACP isoforms are primarily dependant on tissue source.

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CHARACTERIZATION OF THE PYROPHOSPHATE DEPENDENT PHOSPHOFRUCTOKINASE OF *CITRULLUS LANATUS* COTYLEDONS

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During the early stages of seedling growth of *C. lanatus* the PPi:D-Fructose-6-phosphate 1-phosphotransferase (PFP) activity in the cotyledons sharply increases and peaks at the stage of maximum gluconeogenesis. Throughout germination and seedling establishment, PFP activity is present in predominantly two forms eluting at 0.14 M and 0.18 M on an ion exchange column, respectively. During maximum gluconeogenesis most of the PFP activity is present in the form eluting at 0.18 M KCl. After the gluconeogenic burst the PFP activity of the form eluting at the higher conductivity, decreases substantially resulting in a major change in the ratio between the two forms. The molecular weight and kinetic properties of the PFP isoenzymes differ. Protein blot analysis indicates that the form eluting at the higher conductivity is a homomer while the other form is a heteromer.

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SYNERGISTIC INHIBITION OF PISUM PYRUVATE DEHYDROGENASE KINASE BY PYRUVATE AND ADP

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The effects of various metabolites on pyruvate dehydrogenase (PDH) kinase catalysed inactivation of the pyruvate dehydrogenase complex (PDC) were studied in extracts of mitochondria purified from green leaf tissue of Pisum. Pyruvate was an uncompetitive inhibitor of PDH kinase with respect to ATP whereas ADP was a competitive inhibitor. In the absence of pyruvate, very low ATP/ADP ratios of less than 0.2 were necessary to inhibit the kinase and thus favour activation of PDC. Pyruvate substantially raised the ATP/ADP ratio at which inhibition was observed. Inhibition of PDH kinase by pyruvate and ADP was synergistic and the addition of ADP changed pyruvate from an uncompetitive inhibitor to a non-competitive inhibitor. This result indicates that pyruvate inhibits PDH kinase by acting as a "dead-end" inhibitor which binds to the PDH kinase-ADP reaction intermediate.

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METABOLISM OF FATTY ACID HYDROPEROXIDES BY SUNFLOWER PLANTS

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Plants have two pathways for the metabolism of fatty acid hydroperoxides, which are produced by the action of lipoxygenase on polyunsaturated fatty acid. In one pathway, hydroperoxide dehydrase (HD) leads to the biosynthesis of jasmonic acid. In the other pathway, hydroperoxide lyase (HL) leads to the formation of hexanal or hexenal and 12-oxo-dodecenoic acid. The functions of these metabolites in plant metabolism is not clear. To provide more knowledge about the roles of these pathways, various organs of sunflower, Helianthus annuus, were examined for the presence of the two enzymes. Both GC and HPLC were used to detect the products of hydroperoxide metabolism. In the roots of three-week-old, light-grown seedlings, HD accounted for approximately 75% of the hydroperoxide metabolism, and HL 25%. The hypocotyl and cotyledons also had high activity of HD, and constituted about 90% of the hydroperoxide metabolism. In the first true leaves, HL was the only enzyme of hydroperoxide metabolism detected. The results demonstrate that the metabolism of fatty acid hydroperoxides varies markedly among plant organs.

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CITRATE SYNTHASE IN NON-GLYOXYSOMAL PEROXISOMES

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Peroxisomes perform the ß-oxidation in higher plant cells. Non-glyoxysomal peroxisomes, i.e. all peroxisomes except glyoxysomes, lack the glyoxylate cycle (isocitrate lyase and malate synthase) to metabolize the acetyl-CoA formed by ß-oxidation, and the fate of the acetyl-CoA has still to be elucidated. An acetyl-CoA hydrolase was not detected in the non-glyoxysomal peroxisomes from potato tuber but a citrate synthase (CS). CS of peroxisomes (CS I) and mitochondria (CS II) were separated by chromatography on hydroxylapatite. CS I was inhibited with t 1/2 = 30 s by 50 µM 5,5'-dithiobis-2-nitrobenzoate (alike glyoxysomal CS) while CS II was not affected. 25 µM palmitoyl-CoA inhibited CS II but not CS I. Two CS were also obtained from avocado mesocarp which contains non-glyoxysomal peroxisomes. The properties of the two avocado CS correspond to those of CS I and CS II of potato tuber. Our results suggest that, in non-glyoxysomal peroxisomes, the acetyl-CoA is metabolized to citrate.

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MODIFIED BRANCHED-CHAIN AMINO ACID PATHWAYS GIVES RISE TO ACYL ACIDS OF TOBACCO EXUDATE SUCROSE ESTERS

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A major diversion of carbon from branched-chain amino acid biosynthesis/catabolism to form acyl acid substituents of sucrose esters has been observed to be associated with trichome head cells which secrete sucrose esters. Results of radiolabeling experiments indicate that 3 methylvaleryl and 2 methylbutyryl groups are derived from the threonine path of branched-chain amino acid metabolism, that 3 methylbutyryl and methylpropionyl groups are formed via the pyruvate path, and that acetyl groups are derived directly via acetyl CoA. Preliminary evidence was obtained for involvement of cycling reactions in acid chain-lengthening and for utilization of 2-oxo-butyrate to form straight chain acyl substituents.

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GENETIC AND BIOCHEMICAL ANALYSIS OF SUCROSE ACCUMULATION IN TOMATO FRUIT.

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Fruit of domesticated Lycopersicon esculentum accumulate fructose and glucose in approximately equal levels whereas fruit of Lycopersicon chmielewskii accumulate sucrose. The accumulation of sucrose in L. chmielewskii is associated with low levels of both invertase and sucrose synthase. F1 hybrids of the two species accumulate hexose whereas, in F_2 population, the trait of sucrose accumulation segregates in a ratio of approximately 1:16, suggesting that recessive gene(s) determine this trait. Sucrose-accumulating individuals were also identified in a back-cross F_2 population. In these individuals invertase levels were low but sucrose synthase levels high. Analysis of invertase isozyme levels in sucrose accumulating fruit will be presented.

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RESPONSES TO HEAT SHOCK, ARSENITE, AND CADMIUM IN SOYBEAN

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Heat shock (HS), arsenite (As) and cadmium (Cd) treatments induced the 'HS response' in soybean seedlings but differed in their abilities to induce stress tolerance. Pretreatment of seedlings with sub-lethal HS protected them from subsequent normally lethal HS treatment. However, the protection was much more pronounced in 1 day-old than in 2 day-old plants. Sublethal arsenite pretreatment resulted in only a low level of protection against lethal As or HS treatment and severe damage still occurred in specific tissues. Cadmium did not induce any self- or cross-protection. DNA sequence analyses revealed that HS, As and Cd induced the transcription of similar sequences. However, Northern blot analyses of HS mRNAs, and analyses of in vitro translation products and in vivo-labeled proteins by 1D and 2D SDS-PAGE demonstrated that, compared to HS, the response to the chemical stresses was slower, less intense and not as selective. Apparently any causal relationship between HS proteins and induced stress tolerance must also involve developmental-, tissue-, and/or quantitative-specificities.

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GENE EXPRESSION DURING WOUND SHOCK IN LEAF SEGMENTS OF C_3 and C_4 PLANTS

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The wound response in two-week-old C_3 (peanut and soybean) and C_4 (sorghum and corn) plants was followed in leaf segments (5x3 mm) labelled for 2 h with [^{35}S]methionine at 0,2,4 or 6 h after cutting. Absorption of the radiolabel and its subsequent incorporation into protein reached steady-state levels within 4 to 6 h. The high molecular weight proteins associated with the stress response were induced both by cutting and by exposure of the leaves to high temperatures (40° and 45°C). In sorghum and corn, cutting also increased the synthesis of the 98 and 102 kD forms of phosphoenolpyruvate carboxylase, but only the latter form was stimulated by high temperature. Although several low molecular weight polypeptides were synthesized in response to heat shock, they were not induced in any of the four species by wounding. The control mechanisms involved in the transient wound response are currently under investigation.

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THE EFFECT OF LOW TEMPERATURE STRESS ON PROTEIN SYNTHESIS IN COLD TOLERANT AND COLD SENSITIVE TOMATO (LYCOPERSICON ESCULENTUM).

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Siberian, a highly cold tolerant commercial variety of tomato, can not only survive a cold shock of 4°C but grows and fruits at this temperature. Our results indicate marked differences between the cold-stress polypeptide profiles of Siberian and a common cold-sensitive variety, Beefsteak; these differences were recorded both after a rapid cold shock (to 6°C) and from a gradual (2°C/day) downshift in temperature (from 21°C to 6°C). When the polypeptide profiles from the cold shock and the gradual downshift were compared in each variety, these profiles showed 8 common stress proteins in Beefsteak and 13 in Siberian. We suggest a relationship may exist between the ability of the plant to produce specific cold-shock proteins and the increased resistance to chilling temperatures. (Supported by NSERC).

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EFFECTS OF CHILLING ON THE EXPRESSION OF LHCP-II IN CHILLING-SENSITIVE AND -INSENSITIVE PLANTS

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The chilling-sensitive species tomato shows altered patterns of protein synthesis subsequent to a brief exposure to chilling temperatures. One significant change is in the synthesis of the major chl a/b binding protein of PS II, LHCP-II. Using *in vivo* labelling techniques it can be seen that in unchilled control plants, LHCP-II is among the most abundantly synthesized proteins in the leaf. However during the first 2h after chilling, synthesis of LHCP-II declines, reaching undetectable levels after 8h at room temperature in the dark. Another chilling-sensitive species, cucumber, also shows altered synthesis of LHCP-II in response to chilling treatment. Like tomato, synthesis is observed during the first 2 h of the rewarming period following the chill and decreases to undetectable levels after 8 h rewarming. However, in contrast to tomato, no LHCP-II synthesis is detected in the unchilled control plants. Spinach, a chilling-insensitive species, shows no detectable changes in LHCP-II synthesis in response to chilling treatment. Northern blot analyses of tomato and cucumber show that LHCP-II transcript levels decline along with the rate of net synthesis in the chilled plants.

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COLD ACCLIMATION-SPECIFIC GENE EXPRESSION IN CELL SUSPENSION CULTURES OF ALFALFA. L.A. Wolfraim and R.S. Dhindsa, Biology Dept., McGill Univ., Montreal, Quebec, Canada H3A 1B1

In a previous study we demonstrated cold acclimation-specific (CAS) gene expression for alfalfa seedlings, which was positively correlated with the freezing tolerance of four cultivars of alfalfa (Plant Physiol. 89: 375-380). We now provide evidence that the expression of at least one family of CAS sequences is similarly induced during cold acclimation of cell suspension cultures of a freezing tolerant cultivar, Medicago falcata cv. Anik. Expression is rapid from as early as 5h increasing until 4 days. Transcript abundance was higher in CA linear cultures than in CA stationary phase cultures. Cold temperature alone, without exogenous ABA, is sufficient to induce expression and to promote cold acclimation of Anik cell suspension cultures. Nuclear run-on experiments indicate that expression is regulated, at least in part, at the level of transcription and that the gene is transcribed by RNA polymerase II. An increase in ribosomal DNA transcription was also observed.

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COLD ACCLIMATION INDUCED CHANGES IN FREEZING TOLERANCE AND TRANSLATABLE mRNA CONTENT IN CITRUS

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Cold acclimation induced changes in freezing tolerance and gene expression were compared in the relatively cold sensitive pummelo *(Citrus grandis* (L.) Osbeck) and cold hardy trifoliate orange *(Poncirus trifoliata* (L.) Raf.). Seedlings, 2 to 4 months old, of both species were cold acclimated for 4 - 8 weeks at 5°/5°C (day/night) for trifoliate orange and 10°/5°C for pummelo (pummelo exhibited chilling induced chlorophyll bleaching at constant 5°C). Nonacclimated seedlings were maintained at 25°C. LT_{50} values were calculated based on both visual observation and electrolyte leakage following controlled freezes of intact seedlings (-2°C/hr) in a polyethylene glycol bath. Cold acclimation of pummelo resulted in a decrease in LT_{50} from -6°C to -9°C upon cold acclimation, while in trifoliate orange, the LT_{50} decreased from -7°C to less than -19°C. Qualitative changes in *in vitro* translation products, revealed by 2-D PAGE, were observed following cold acclimation in both species. An mRNA for a large protein (ca. 165 Kdal, pI 5) induced during cold acclimation in trifoliate orange was not detected in cold acclimated pummelo.

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IDENTIFICATION OF COLD-INDUCIBLE GENES CODING FOR HEAT-STABLE POLYPEPTIDES IN ARABIDOPSIS THALIANA

Chentao Lin, Ravindra Hajela, David Horvath, Sarah Gilmour, and Michael Thomashow, Department of Crop & Soil Sciences, Michigan State University, East Lansing, Michigan 48824

Arabidopsis thaliana becomes more tolerant to freezing temperature when first exposed to low but nonfreezing temperature (Gilmour et al. (1988) Plant Physiol. 87:745-750). During this cold acclimation period, a group of new mRNAs are induced whose translation products are heat-stable. Three cold-induced cDNA clones were found by hybrid selection to encode heat-stable polypeptides of 160 kD, 47 kD and 15 kD. The expression of these genes and the heat-stability features of their gene products will be discussed.

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GENE EXPRESSION IN ARABIDOPSIS THALIANA IN RESPONSE TO LOW TEMPERATURE

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Many plants have the ability to acclimate to cold temperatures. Arabidopsis thaliana increases its frost tolerance after a period of low, non freezing temperature. During this cold treatment several new proteins and mRNA species accumulate (Gilmour et al. (1988) Plant Physiol. 87: 745-750). To examine the cold acclimation process at the molecular level, we have isolated, by differential screening of a cDNA library, five cDNA clones whose mRNAs accumulate at low temperature. One of these, pHH28, encodes a 160 kD, pI 4.5 polypeptide which accumulates in the cold. Two other cDNA clones, pHH29 and pHH67, were found to encode 20 kD and 18 kD polypeptides, respectively. Initial characterization of these cDNA clones will be described.

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CHARACTERIZATION OF BRASSICA NAPUS MICROSPORE DEVELOPMENT IN THE ANTHERS BY FLOW CYTOMETRY.

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Microspore culture is an important technique used for the production of doubled haploids in rapeseed breeding programs. Only microspores in the late uninucleate stage respond to the culture conditions and develop into embryos. By flow cytometry light scatter properties of large numbers of cells can be determined. This technique was used to characterise the properties of microspore cells from B. napus (cv Topas) buds at early, middle and late stages of development. Maturation of the microspores was accompanied by changes in their forward angle light scatter (FALS, related to cell size) and 90° light scatter (90°LS related to granularity) properties. In particular, samples of cells from young buds had two populations of cells that could be distinguished on the basis of their 90° LS properties. Sorting experiments showed that the population with high 90°LS values consisted predominantly of tetrads and the population with lower 90°LS values consisted of single cells. In samples from older anthers the 90°LS profile contained a single peak. However, in these samples the FALS signal was split into two. The two populations represent microspores at different stages of maturation.

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MICROSPORE CULTURE AS AN EFFICIENT SYSTEM FOR TRANSFORMATION IN BRASSICA NAPUS

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Microspore culture in Brassica napus is one of the most efficient system of in vitro embryogenesis in plants. Under optimal conditions, up to 50% of the cultured microspores develop into embryoids of which more than 80% remain haploid. Transformation of uninucleate microspores and subsequent chromosomal doubling lead to the production of homozygous diploid transformants. In order to facilitate micromanipulation, low density cultures in conjunction with feeder layers were established in which the efficiency of microspore embryogenesis was enhanced by up to 10 fold. Plasmids containing the coding region for beta-glucuronidase (GUS) were delivered into microspores by microinjection. A high proportion of the embryoids/plantlets derived from injected microspores expressed GUS activity. Transformation of microspores via electroporation will be discussed, as well as transformation of protoplasts originated from cell cultures of microspore-embryoids by microinjection and electroporation.

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ELICITATION OF SESQUITERPENE PHYTOALEXIN BIOSYNTHESIS IN TRANSFORMED ROOT CULTURES OF *HYOSCYAMUS MUTICUS* L.

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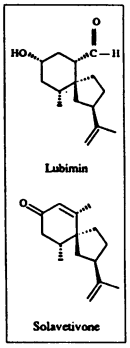
"Hairy root" cultures of *Hyoscyamus muticus* L. (Egyptian henbane), established over 5 years ago, continue to show stable growth and tropane alkaloid production. These cultures produce and release large amounts of the fungitoxic sesquiterpenes lubimin and solavetivone into the liquid culture medium upon elicitation with a mycelial extract from the soil pathogen *Rhizoctonia solani*. The kinetics of this response is similar to previous reports on phytoalexin elicitation in Solanaceae cell suspension cultures, and results in a reduction of fresh and dry tissue weight, accompanied by tissue browning. Elicited roots resume normal growth upon transfer to fresh medium and can be re-elicited. The production of hyoscyamine, a tropane alkaloid, normally occurs in these root cultures at concentrations similar to those found in the intact plant. Alkaloid content per dry weight also increases upon elicitation, but this may be an indirect result of changes in growth caused by elicitor addition. The effect of culture parameters and inhibitors of the terpenoid pathway on both sesquiterpene and tropane alkaloid production are being investigated.



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CELL CULTURES FROM AGROBACTERIUM RHIZOGENES TRANSFORMED PAPAVER SOMNIFERUM

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Infection of P. somniferum seedlings with various strains of A. rhizogenes has shown strain variation in the induction of adventitious roots. Culture of the adventitious root has lead to the establishment of numerous cell lines. All adventitious root derived cell lines are phytohormone autotrophic, produce opines, accumulate benzylisoquinoline alkaloids and regenerate under specific culture conditions. Investigations of alkaloid accumulation in these transformed lines show the major alkaloid to be a benzophenanthridine, sanguinarine, which can be induced to accumulate to levels > 2.5 % DWT. The regeneration process can be manipulated by altering media composition or culture age.

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EFFECTS OF CYTOCHALASIN D ON THE ORGANIZATION OF ACTIN MICROFILAMENTS DURING POLLEN GERMINATION IN PYRUS COMMUNIS L.

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To elucidate the functional significance of the changes in actin organization (see Protoplasma 147:5-15: 1988), we administered cytochalasin D (CD) to germinating pollen in the following ways: 1. pollen grains were directly exposed to CD for 2-45 minutes; 2. pollen grains were allowed to undergo normal activation up to various stages of actin organization and then exposed to DC; and 3. pollen grains at different stages of actin organization were exposed to short CD pulses and then allowed to develop normally. The organization of actin in the treated pollen was monitored via staining with rhodamine-phalloidin. The results indicate that although CD disrupted the normal organization of actin microfilaments, it did not affect the organized movement of actin. CD also inhibited pollen germination. It is concluded that the maintenance of a dynamic integrity in the organization of actin microfilaments is essential for pollen germination.

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PHYSIOLOGICAL MARKERS OF SOMATIC EMBRYO INDUCTION AND DEVELOPMENT IN ALFALFA.

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Using a model forage crop system developed with Medicago sativa L. cv Rangelander, we have shown that the ability to form somatic embryos in vitro is a dominant trait that can be transferred by simple sexual crossing. Large yeilds of viable dried somatic embryos can be produced in about 8 weeks and plants recovered after extended periods of storage. Results show that somatic embryos are usually biochemically immature at the time of "germination". For example, storage protein content is only about 10 % of that of a mature seed. However, some aspects of embryo maturity can be manipulated in vitro to partially overcome some of the observed deficiencies.

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GERMINATION OF DESICCATED SOMATIC EMBRYOS OF ALFALFA (MEDICAGO SATIVA L.).

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Large quantities of somatic embryos were obtained in a synchronize manner from alfalfa and treated to induce desiccation tolerance. The embryos were dried to a low moisture content (10%). Upon imbibition, there was a rapid uptake of water into the embryo. They germinated in a morphological manner analogous to a true seed and developed into normal plants. However, a reduction in the rate of water uptake reduced the leakage of cytoplasmic components during imbibition and enhanced seedling vigour, which indicates that the lack of a seed coat in somatic seeds is a disadvantage. Comparative studies of reserve mobilization of somatic seeds and "true" alfalfa seeds were also made to understand the germination process to further improve seedling vigour of the artificial seeds.

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MOLECULAR ANALYSIS OF ASYMMETRIC SOMATIC HYBRIDS USING SPECIES-SPECIFIC REPETITIVE DNA PROBES

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Asymmetric somatic hybrids (containing the entire genome of a recipient species plus a limited amount of genetic material from a donor species) provide a unique approach for plant gene transfer. However assessing the practical utility of asymmetric hybrids requires methods for quantifying the amount of donor genetic material in the hybrids and determining whether this material is organized as intact chromosomes or as translocations. We have prepared asymmetric hybrids of *N. tabacum* (*Nt*) + *N. plumbaginifolia* (*Np*) by fusing *Nt* protoplasts with γ-irradiated *Np* protoplasts. Plants regenerated from these fusions have been analyzed using several species-specific, chromosomally dispersed, repetitive DNA probes isolated from *Np*. Dot blot hybridization shows some of the hybrids retain 1-5% *Np* DNA whereas others retain 20-30% of the *Np* genome. *In situ* hybridization with these probes indicates that some of the hybrids retain only a single *Np* chromosome, some retain several *Np* chromosomes, and some have intergenomic translocations.

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PHENYLPROPANOID METABOLISM IN FUNGAL CHALLENGED TOMATO CULTURES

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A co-cultivation system has been developed to study the tomato-Verticillium albo-atrum interaction in vitro. V. albo-atrum grows more slowly in co-cultivation with cultures derived from Verticillium-resistant (Ve^+) plant tissue than with cultures derived from Verticillium-susceptible (Ve^-) plant tissue. Fungal challenged Ve^+ cultures show an early induction of measurable phenylalanine ammonia-lyase (PAL) activity as well as an accumulation of soluble phenolics. Ve^- cultures, on the other hand, display a delayed PAL induction and no soluble phenolic accumulation. HPLC analysis of soluble phenolics indicates the presence of p-coumaric, caffeic and ferulic acids in Ve^+ extracts post challenge. The presence of these lignin biosynthesis precursors is consistent with the deposition of lignin-like coating materials in whole plants challenged with V. albo-atrum.

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PROTOPLAST-FUSION DERIVED CYBRIDS ILLUMINATE NUCLEAR-ORGANELLE INTERACTIONS IN SOLANUM

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To study the compatibility between a given nuclear genome and alien organelles we established cybrids between potato and several Solanum species. The latter were utilized as donors of mitochondria and chloroplasts in donor-recipient protoplast-fusion systems to obtain cybrids with potato nuclear genomes but alien organelles. For organelle donors we chose different Solanum species, e.g. S. chacoense, S. brevidens, S.etuberosum and S. berthaultii. All but the combination of potato with S. brevidens, resulted in green cybrid plants. The latter developed up to pale shooty cultures. Organelle compositions were evaluated by the restriction profiles of their respective DNA, supplemented by Southern blot hybridizations with appropriate organelle DNA fragments. Transfer of organelles from Solanum species to potato varied considerably, providing information on organelle phylogeny and on nuclear/organelle-genome capabilities.

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MEMBRANE FLUIDITY CHANGES IN CULTURED CELLS GROWING ON AUXIN-FREE MEDIUM

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Using fluorescence polarization we have demonstrated that membrane fluidity changes occur after cultured plant cells are transferred from auxin-containing to auxin-free nutrient medium. An embryogenic cell suspension of Asclepias tuberosa was derived and maintained on a Murashige Skoog (MS) medium supplemented with 5 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D) to inhibit embryogenesis. To initiate experiments cultures were washed and resuspended in 500ml MS medium either with or without 2,4-D. Protoplasts were prepared and membrane fluidities determined by the membrane interior probe DPH or the aqueous interface probe TMA-DPH. The DPH probe indicates that initially membranes of cells removed from 2,4-D become less fluid relative to cells maintained on 2,4-D, but after day 4 become significantly more fluid. No differences were observed using the TMA-DPH probe.

814

MOLECULAR CHARACTERIZATION OF THE PHOTOSYNTHETIC MUTATION *Su* IN *Nicotiana tabacum*.

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We have characterized the semidominant nuclear aurea mutation *Su* in tobacco by analyzing the expression of several nuclear (nuc)- and chloroplast (ct)-encoded photosynthetic genes at both the RNA and protein level. Samples were derived from the homozygous mutant *Su/Su* (albino) the heterozygous *Su/+* (yellow-green) and the wild-type +/+ (green) plants. Northern blot analysis of RNA from leaf tissue of heterotrophically-grown plants revealed two patterns of accumulation. For the mRNAs that encode cytochrome b559 (ct-encoded), the 32 KD quinone-binding protein of PS II (ct-encoded), and the large (ct-encoded) and small (nuc-encoded) subunits of rubisco, the steady state levels of RNAs were comparable in the specified plant types. In contrast, the light-harvesting chlorophyll protein (LHCP) (nuc-encoded) mRNA level in the homozygous mutant was substantially diminished in relation to levels found in the heterozygote and wild-type. In addition, the circadian pattern of LHCP mRNA accumulation appeared to be different in *Su/Su* plants. Based on western blot data, the proteins encoded by all the aforementioned genes are reduced in the homozygous mutant. LHCP in the heterozygote was found to be less than that in the wild-type. In conclusion, while it is apparent that this mutation affects the level of LHCP mRNA, we have not yet determined whether this effect is due to abnormal turnover rates or aberrant transcriptional control. Nevertheless, the results indicate that the *Su* mutation does have an effect on post-transcriptional regulation for all genes examined. Furthermore, the *Su* mutation effects are not restricted to the nuclear-encoded components of the photosynthetic apparatus but the effects extend to chloroplast-encoded components as well. Moreover, this mutation differentially affects the expression of nuclear genes such as the small subunit of rubisco and LHCP.

815

PHYTOCHROME REGULATION OF 5-AMINOLEVULINIC ACID (ALA) IN DEVELOPING CHLOROPLASTS

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When dark grown cucumber seedlings, previously exposed to white light for 20 h, were returned to darkness, the ability of isolated chloroplasts to synthesize ALA dropped by about 70%. The seedlings were then exposed to light, and the synthetic ability of the isolated chloroplasts was determined. Restoration of the synthetic capacity was promoted by continuous white or red light or by intermittent red light. Blue and far-red light were ineffective. Blue light after a red pulse did not enhance the effect of the red light. Far-red light given immediately after each red pulse prevented the stimulation due to intermittent red light. Restoration of the biosynthetic activity by *in vivo* light treatments was inhibited by cycloheximide. These findings suggest that the majority of the plastidic ALA synthesis is under phytochrome regulation, involving translation on 80 S ribosomes. NSF support.

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MEMBRANE-ASSOCIATED PHYTOCHROME: ASSOCIATION WITH MEMBRANES AND DISTRIBUTION IN TISSUES. William Eisinger[1], Timothy W. Short[2] [1] Santa Clara University, Santa Clara, CA 95053; [2]Carnegie Institution of Washington, Plant Biology Department, 290 Panama Street, Stanford, CA 94305.

Phytochrome associated with cell membranes comprises less than 10% of the total phytochrome in developing third internodes of etiolated pea seedling epicotyls. When phytochrome was extracted from isolated membranes using Triton X-114 phase separation, phytochrome was found in the aqueous phase and not in the detergent phase. Thus, this phytochrome fraction is hydrophilic. Membrane-associated phytochrome was found at approximately constant levels (per mg total protein) throughout the pea epicotyl. However, soluble phytochrome was 80% lower in nongrowing regions. Soybean hypocotyl tissues contain high levels of membrane-associated phytochrome. As with pea, this phytochrome declined with red light exposure; a 4 hr exposure resulted in a 90% reduction. Incubation of excised hypocotyl segments with ^{14}C-palmitic acid did not result in label associated with phytochrome. Thus, it appears unlikely that phytochrome proteins are acylated by the fatty acid as a mechanism of attachment to membranes. Membrane-associated phytochrome was not found in soybean cells grown in liquid suspension culture. The possible physiological role of membrane-associated phytochrome will be discussed.

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IDENTIFICATION AND PARTIAL CHARACTERIZATION OF A PUTATIVE RED ALGAL PHYTOCHROME

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We have begun a study to identify and characterize a phytochrome molecule from a red alga. The rationale for this undertaking is twofold: 1) Identification of conserved regions of a "primitive" molecule will identify domains essential for function. 2) Characterization of a marine phytochrome will elucidate how photoperception can occur underwater, a light environment drastically different than terrestrial light environments. Using the red alga Audiounella botryocarpa as a model system, we have identified a protein which is recognized by monospecific polyclonal anti-phytochrome (etiolated oat) antibodies. Thus, this protein shares at least one epitope with etiolated oat phytochrome. We have shown after partial purification that this algal "phytochrome" is an acidic 96-kDa protein which behaves as a monomer in size exclusion chromatography.

818

CHANGES IN INDOLE-3-ACETIC ACID AND EXTENSIBILITY DURING DE-ETIOLATION OF PEAS EXPOSED TO RED LIGHT.

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Dark grown pea stems irradiated with red light begin a decline in extension rate after one hour and reach a maximal inhibition after 3 hours as measured with displacement transducers. Previous kinetic studies have shown that genotypes with differing gibberellin levels and sensitivity respond similarly to red light. Therefore an analysis of IAA levels was undertaken. Transducers were used to locate regions of the epicotyl that are responsive to red light. IAA was extracted from these regions, purified by HPLC and quantitated by GC-MS. Extensibility in the light responsive regions was measured by creep analysis. Plastic and elastic extensibility decline in red light-treated epicotyls within 3 hours, however preliminary work suggests that changes in IAA levels in whole stem tissue and epidermis from red light responsive regions are not significant.

819

BEAN LEAF GROWTH IN LIGHT IS REGULATED BY PHYTOCHROME AND A BLUE-LIGHT RECEPTOR

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Cell expansion in primary Phaseolus vulgaris leaves has been characterized with respect to requirements for light and ions. Continuous exposure to light of relatively high fluence rates is necessary for bean leaf cell growth. Blue (460 nm) and red (660 nm) light are most effective. Results from leaves bleached with tentoxin, or photosynthetically-inhibited by DCMU, indicate that chlorophyll is not the pigment mediating this growth response. Light hyperpolarizes the epidermal cell membranes ± DCMU. Both phytochrome and a blue-light receptor are involved. Leaf cell growth in white light is highly and specifically dependent on external K. Elevated K will not stimulate growth in the dark. The requirement for K by cells growing in response to either red or blue light is being tested.

820

EFFECTS OF BLUE LIGHT ON RAPID PHOSPHORYLATION OF A PEA PLASMALEMMA PROTEIN

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As reported previously, we have observed that *in vitro* phosphorylation of a specific plasma membrane protein from etiolated pea stem sections is affected by *in vivo* blue light irradiation. We have further characterized this reduction in phosphorylation after a light pulse in order to determine whether a correlation exists between the biochemical effect we have observed and known physiological blue light responses. The phosphorylation is strongest in the growing region of epicotyls, diminishes in more basal tissues, and is absent in buds. Fluence response curves varying either time or fluence rate indicate that the phenomenon has a threshold at a fluence of 0.1-0.3 μmol·m^{-2}. The response is saturated by fluences between 100 and 300 μmol·m^{-2}. Reciprocity is valid throughout the range tested. If the stem segments are given a saturating pulse of light followed by a period of darkness prior to membrane isolation, the capacity for phosphorylation returns over a period of 20 to 60 minutes. These findings are correlated with reports in the literature for the first positive phototropic curvature of seedlings, indicating that the phosphorylation could be a step in the signal transduction chain for that response.

821

REGULATION OF rRNA GENE EXPRESSION DURING LEAF DEVELOPMENT

Scott R. Baerson , Kenneth J. Piller , & Lon S. Kaufman Dept. of Biol. Sci., LCMDB, Univ. of Il. at Chicago, Chicago, Il. 60680

We have examined the rate of rDNA transcription during pea leaf development. Nuclear run-on assays were conducted using apical tissue from 7-day-old seedlings exposed to 0 through 7 days of white light. rDNA transcription rates in seedlings exposed for one day are 70% greater than dark-grown seedlings. Two and 3 days of exposure results in rates approximately 2.5-fold greater than dark-grown seedlings. Four through 7 days of exposure results in dark-grown rates. The increased rate on days 2 and 3 does not correlate with a change in ploidy or an increase in rDNA copy number. The rates of total and Cab transcription also increase during the first two days of light exposure, but do not return to dark-grown rates as does rDNA. These results indicate that rDNA transcription is increased during specific stages of leaf development. Supported by USDA grant #86CRCR12228 to LSK.

822

TWO BLUE LIGHT RESPONSES REGULATE BOTH MOLECULAR AND PHYSIOLOGICAL EVENTS.

K.M.F. Warpeha, K.A. Marrs & L.S. Kaufman. Univ. of Ill. at Chicago, Dept of Biol. Sci., LCMDB, Chicago, IL 60680

We have examined the blue-light, fluence-response characteristics for accumulation of four transcripts and several physiological parameters in both red-light-grown and dark-grown pea seedlings. Data obtained from red-light grown seedlings indicate two blue light responses, one with a threshold to low fluences of blue light and a second with a threshold to higher fluences. Cab RNA, pEA215 RNA and supression of epicotyl elongation show both responses, pEA25 RNA and chlorophyll and carotenoid accumulation show only a low fluence response and pEA207 RNA shows only a high fluence response. Growth in darkness eliminates the high fluence responses observed for Cab, pEA215 and pEA207 RNAs and supression of epicotyl elongaion. Growth in red light cannot be replaced by a single, short pulse of red light immediately preceeding the blue-light treamtment.

Supported by USDA grant # 866CRCR122228 to LSK.

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BLUE-LIGHT REGULATION OF NUCLEAR GENE EXPRESSION IN PISUM SATIVUM.

K.A. Marrs, K.M.F. Warpeha, and L.S. Kaufman, University of Illinois at Chicago, LCMDB, Chicago, IL 60680.

We are examining the role of blue light in the regulation of nuclear gene expression in pea. Seedlings receiving a single pulse of blue light show an increase in the RNA level and transcription rate of the Cab (Chlorophyll a/b binding protein) gene family and the transcription rate of the ribosomal RNA genes, and a decrease in the RNA levels and transcription rate for two gene families represented by cDNA clones pEA25 and pEA207. We have examined the time-course and fluence-response characteristics of these changes in transcription rate. A comparison of the steady state level of Cab RNA and Cab transcription rate indicate an increase in the degradation rate of Cab RNA in response to high fluences of blue light.

Supported by USDA grant #866CRCR122228 to L.S.K.

824

STRUCTURE-FUNCTION STUDIES OF *AVENA* PHYTOCHROME: MAPPING OF THE *IN VITRO* PHOSPHORYLATION AND ATP BINDING SITES

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A polycation stimulated protein kinase activity has been found associated with purified *Avena* phytochrome (Wong et. al. 1986 *J. Biol. Chem.* 261:12089). This activity phosphorylates phytochrome within the 10 kDa N-terminal domain *in vitro*. Phytochrome has also been shown to be modified by two ATP affinity analogs (Wong and Lagarias 1989 *Proc. Natl. Acad. Sci. USA* in press), indicating the presence of an ATP binding site in the protein. Further characterization of the association of this protein kinase activity with phytochrome, reported here, was accomplished by mapping both the *in vitro* phytochrome phosphorylation sites and the sites modified by the ATP analogs. The implication of these results toward the understanding of the phytochorme-mediated signal transduction pathway is also discussed.

825

NOVEL MITOCHONDRIAL GENOMES IN *B. NAPUS* PLANTS

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We are studying 11 *B. napus* somatic hybrids with novel mitochondrial genomes produced by protoplast fusion of *ogura* cms *B. oleracea* and atrazine resistant *B. campestris* (Robertson et al, TAG 74:303-309(1987); Jourdan et al, TAG in press). All contain *campestris* chloroplasts. In some, the mitochondrial genome is identical to that of *campestris*. In others it consists of regions from both fusion partners. To determine the regions involved in the novel genomes, we used fragments from about 30% of the *campestris* mitochondrial genome, selected gene-containing fragments from *ogura* cms and male fertile radish, and the coding sequence of the *atp9*-4 gene of petunia (provided by O. Folkerts). The majority of changes occur in the *atp9*, *atp6*, *coxI* and *atpA* regions. The regions between these genes are not rearranged. Both of the novel fragments occur only in the *atp9* region. One includes the *atp9* coding sequence. The *campestris coxI* and *atpA* gene-containing fragments are present in all 11 plants. Some also have the *ogura* derived fragment. 7/11 plants have *ogura atp6*, 3/11 have *campestris atp6* and one plant has both. The transcripts of 7 plants are those predicted except for the plant with both coding regions; it produces only the *ogura* transcript. We are analyzing more plants and mapping the mitochondrial genomes. We hope to find a region associated with cms.

826

HETEROGENEITY IN THE 3'TERMINI OF MESSAGES ENCODING THE LARGEST SUBUNIT OF RNA POLYMERASE II IN ARABIDOPSIS THALIANA

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The Carboxyl Terminal Domain (CTD) is an unusual domain encoded by the 3' terminus of the gene for the largest subunit of RNA polymerase II. The CTD consists of tandem repeats of a 21bp sequence which translates to the heptapeptide: PTSPSYS. The number of repeats in the CTD varies from 26-52 depending on the organism. The Arabidopsis CTD consists of 39 repeats. However, the sequence encoding the Arabidopsis CTD is interrupted by two introns, a feature unique to the plant genes. Polymerase Chain Reaction (PCR) was used to examine the portion of the message encoding the CTD. PCR results indicate the presence of an intron in the 3' untranslated region which is not always removed during processing. We have additional evidence suggesting that heterogeneity is generated in the analogous soybean transcripts by this same mechanism. These introns in the 3' untranslated region may be involved in regulation.

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MOLECULAR CHARACTERIZATION AND IDENTIFICATION OF A SULFUR-RICH SEED PROTEIN AND ITS cDNAs FROM COUROUPITA GUIANENSIS

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The major protein in the seeds of cannonball plant (Couroupita guianensis) is of low molecular weight 2S protein (CB2S). This protein fraction comprises two protein components, CB2S-1 and CB2S-2. Both protein components consist of a large polypeptide and a small polypeptide subunits, having similar mol wt around 9 kD and 3 kD respectively. Amino acid analysis indicates that the CB2S contains exceptionally high levels of the sulfur amino acids, 25.7% Met and 7.56% Cys. The sequence of 11 N-terminal amino acids has been determined for the CB2S. Cell-free translation study indicates that the CB2S is synthesized as a larger (18 kD) precursor polypeptide. Molecular cloning and sequence analysis of cDNAs encoding the CB2S will be presented and discussed in regard to its unusual amino acid composition.

828

STRUCTURE AND EXPRESSION OF TWO MAIZE POLYBUIQUITIN GENES

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We have isolated and sequenced two maize genomic clones (UBI1 and UBI2) encoding the highly conserved 76 amino acid protein ubiquitin. Both genes contain seven contiguous direct repeats of the protein coding region. The deduced amino acid sequence of all 14 repeats is identical and is the same as for other plant ubiquitins. Both UBI1 and UBI2 are expressed constitutively in maize seedlings and are thermally inducible. Both genes contain an intron in the 5' untranslated region which is inefficiently processed following a brief, severe heat shock. The transcription start site of UBI1 has been determined and a "TATA" box and two overlapping sequences homologous with the consensus heat shock element are present in the 5' flanking sequence. We have constructed a transcriptional fusion of 0.9kb of the 5' flanking region and the entire 5' untranslated sequence of UBI1 with the coding sequence of the reporter gene chloramphenicol acetyl transferase. Expression of this construct has been analyzed in electroporated protoplasts.

829

MOLECULAR CLONING AND ANALYSIS OF CDNA ENCODING A PLANT TRYPTOPHAN DECARBOXYLASE: COMPARISON WITH ANIMAL DOPA DECARBOXYLASES

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The sequence of a cDNA clone which includes the complete coding region of tryptophan decarboxylase (E.C. 4.1.1.27) from periwinkle (Catharanthus roseus) is reported. The cDNA clone (1747 bp) was isolated by antibody screening of a cDNA expression library produced from poly A^+ RNA found in developing seedlings of C. roseus. The clone hybridized to a 1.8 kb mRNA from developing seedlings and from young leaves of mature plants. The identity of the clone was confirmed when extracts of transformed E. coli expressed a protein containing tryptophan decarboxylase enzyme activity. The tryptophan decarboxylase cDNA clone encodes a protein of 500 amino acids with a calculated molecular mass of 56,142 Da. The amino acid sequence shows a high degree of similarity with the aromatic L-amino acid decarboxylase (dopa-decarboxylase) and the alpha-methyldopa hypersensitive protein of Drosophila melanogaster. The tryptophan decarboxylase sequence also showed significant similarity to feline glutamic acid decarboxylase and mouse ornithine decarboxylase suggesting a possible evolutionary link between these amino acid decarboxylases.

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THREE-DIMENSIONAL MODELS OF PHOSPHOGLYCERATE KINASE (PGK) FROM WHEAT LEAF CHLOROPLASTS AND CYTOPLASM

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The base sequences of wheat cDNA coding for the chloroplast and cytoplasmic isoenzymes of PGK suggest that the evolution of the two isoenzymes may have involved recombination of their genes (Longstaff, M., Raines, C.A., McMorrow, E.M., Bradbeer,J.W. & Dyer, T.A., in preparation). The derived amino acid sequences have been used to construct models of the two isoenzymes with the programme FRODO on an Evans and Sutherland graphics work station. The published coordinates of yeast PGK (H.C.Watson et al.) in the protein data bank were used as template. PGK is highly conserved with 80% homology of amino acid sequence between the plant isoenzymes while each of them shows 50% homology with yeast PGK. PGK is a monomeric hinge-bending enzyme. The most obvious differences between the main chains of the plant PGK and that of yeast occur in the N-terminal domain and include the absence of a loop at residues 128 to 141. The structural models are being studied with respect to differences observed in the regulation of the two plant isoenzymes and differences between the plant and yeast PGK.

Supported by AFRC grant no. PG29/49.

831

ANALYSIS OF POSTTRANSLATIONAL PROCESSING AND SECRETION OF TOMATO POLYGALACTURONASE IN TRANSGENIC TOBACCO PLANTS.

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Tomato polygalacturonase (PG) is a cell wall enzyme synthesized and secreted in large amounts during tomato fruit ripening. Three isoforms of PG, all derived from a single gene product, are present in ripe tomato fruit. In order to investigate further the posttranslational processing and secretion of this enzyme, we have initiated studies of PG expression in transgenic tobacco plants. Agrobacterium-mediated transfection was used to introduce into tobacco leaf discs a full-length PG cDNA under control of the CMV 35S promoter. Regenerated plants selected for kanamycin resistance and expression of nopaline synthase were analyzed for various aspects of PG expression. Our results indicate that 1) immunologically detectable PG can be isolated from the cell walls of transgenic tobacco plants; 2) the enzyme is electrophoretically and immunologically indistinguishable from that isolated from ripe tomato fruit; 3) the protein is enzymatically active in in vitro assays; and 4) only two of the three PG isoforms found in ripe tomato fruit appear to be present in transgenic tobacco. Further analysis of the localization, processing and in vivo activity of PG in this transgenic system will be presented.

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CHARACTERIZATION OF THE CHALCONE SYNTHASE MULTIGENE FAMILY AND mRNA EXPRESSION IN GERMINATING SOYBEANS

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Chalcone synthase mRNA is induced by light and pathogen infection. The normal pattern of CHS gene expression during plant development has not been examined. Since most research on CHS is concerned with pathogen infection early in development, we have begun to characterize CHS during early seedling development. Genomic clones containing four of the six CHS genes from soybean have been isolated. Each gene is apparently represented by one copy per haploid genome. The DNA sequence for GMchs300 has a coding region of 1167 bp, split by one intron. Using GMchs300 as a probe, the abundance of CHS mRNA levels has been measured in roots, hypocotyls and cotyledons during early stages of plant growth. CHS steady state mRNA levels vary between organs and show a diurnal variation. Therefore, during the course of early seedling growth CHS mRNA levels are temporally regulated and further modulated by stage of development.

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MOLECULAR GENETIC EVIDENCE FOR FUNCTIONAL DOMAINS WITHIN THE *VP1* GENE PRODUCT.

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The *viviparous-1* mutant in maize has pleiotropic effects on seed maturation. Most notably, abscisic acid sensitivity in the embryo is reduced causing vivipary, and anthocyanin pigmentation is blocked in aleurone and embryo tissues. However, certain *vp1* mutant alleles have a dormant/anthocyaninless phenotype. DNA probes were used to examine the structure and expression of wildtype and mutant *vp1* alleles. The wildtype transcript (2500 nt in length) is expressed in embryos and to a lesser extent in endosperm. In typical viviparous/anthocyaninless mutants this transcipt is missing. However, the dormant/anthocyaninless allele (*vp1-McWhirter*) expresses a 3′ truncated transcript (~2300 nt). Analysis indicates that approximately 900 bases of the normal coding sequence is deleted or replaced in the mutant transcript. At the genomic level, this mutant exhibits a 4 kbp insertion near the point of divergence of the transcripts. These results suggest that the C-terminal domain of the *Vp1* protein is specifically required for anthocyanin expression, and that the N-terminal domain, by itself, is at least partly functional in arresting embryo development.

834

THE ISOLATION AND CHARACTERIZATION OF ABA- AND DROUGHT-INDUCED cDNAS IN TOMATO

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Recent work in our laboratory using the ABA-deficient mutant, flacca, and the wild type, cv. Ailsa Craig, has shown that several polypeptides and in vitro translation products are synthesized in response to the drought-induced accumulation of ABA. In order to isolate clones which represent mRNAs that accumulate in response to ABA during stress, we constructed a cDNA library in λ Gem 4 from the mRNA population of stressed wild type leaves. Differential screening of the library using ^{32}P reverse transcripts from stressed wild type and stressed mutant mRNA populations allowed for the selection of several putative ABA- and drought-induced cDNAs. Northern analyses of RNA extracted from leaves of flacca and Ailsa Craig, which had been drought stressed or kept turgid, revealed that we had isolated three drought-induced cDNAs. Further Northern analyses using both flacca and Ailsa Craig leaves treated with ABA suggests that these three cDNAs represent mRNAs that are ABA-induced during drought stress.

835

ANAEROBIC TREATMENT ALTERS THE CELL-SPECIFIC EXPRESSION OF Sh, AND Sus GENES IN ROOTS OF MAIZE SEEDLINGS, Jeannie Rowland, Yen-Ching Chen, and Prem Chourey, USDA/ARS, Plant Pathology Department University of Florida, Gainesville, FL 32611

We have examined the in situ expression pattern of Sh and Sus which encode sucrose synthase isozymes SS1 and SS2 respectively in the lower region of the primary root in response to anaerobiosis. In situ hybridization and immunolocalization experiments revealed a unique spatial pattern of expression for the two genes. Induction of Sh was marked by highly elevated SS1 RNA levels in the vascular elements, pith, and epidermis. A significant but less drastic increase in SS protein was found in these same tissues as well as the root cap; the increased level of immunosignal was, however, restricted to cells in about 1 centimeter of the root apex. The specific response of the Sus gene to anaerobic stress was determined using a sh deletion mutant; Sus responded with a slight reduction in SS2 RNA and protein levels except in the root cap where SS2 protein, but not SS2 RNA, was induced. These data indicate that multiple regulatory controls including cell-specific post-transcriptional mechanisms modulate SS levels in anaerobically-stressed seedlings.

836

BACKGROUND-ASSOCIATED VARIATION IN TRANSCRIPTS PRODUCED FROM A Mu1-Adh1 MUTANT ALLELE OF MAIZE

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Mutations induced by insertion of the maize transposable element Mu1 into the first intron of Adh1 decrease the level of gene expression, measured from ADH allozyme ratios. From a DNA sequence comparison of a primary mutant allele and a low-expressing "derivative", we have determined that the alleles are identical, despite their reported three-fold difference in ADH1 levels (Freeling, Cheng and Alleman Dev. Gen. 3, 1982); the alternative phenotypes are dependent on the genetic background in which they are measured. This background-associated variability appears to reflect a bias in patterns of transcripts produced from the mutant allele: in "high ADH" backgrounds, seedlings produce predominantly normal-length transcripts while "low ADH" backgrounds are associated with high levels of aberrant transcripts carrying sequences from Mu1 and IVS1. Clones derived from cDNA libraries are being sequenced to help us define the mechanisms by which the aberrant transcripts are produced.

837a

MOLECULAR GENETIC MANIPULATION OF SECONDARY METABOLISM

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Recent advances in plant molecular biology have led to the potential ability to modify secondary metabolism in ways not possible by traditional breeding and selection. The genes encoding enzymes in several secondary metabolic pathways have been cloned and their regulation is being investigated in many laboratories. Efficient gene transfer systems have been developed for many plants, allowing the reintroduction of such genes. Progress in the use of these genes for manipulation of secondary metabolism will be discussed, including the following areas: gene introduction to create new pathways, manipulations to direct expression of desired pathways in an organ or developmental specific manner, and inhibition of the expression of endogenous genes encoding enzymes of secondary metabolism.

837b

GENETIC MANIPULATION OF FLAVONOID BIOSYNTHESIS IN FLOWERING PLANTS

G. Forkmann

Abstract not available at press time.

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OLIGOSACCHARINS REGULATE TOBACCO THIN-CELL-LAYER MORPHOGENESIS

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We have modified and characterized a tobacco thin-cell-layer (TCL) morphogenesis bioassay for use as a sensitive system to test plant cell wall fragments for morphogenesis-regulating activity. Pectic cell wall fragments were released from suspension-cultured Acer pseudoplatanus (sycamore maple) cells by treatment with endopolygalacturonase from Aspergillus niger. The pectic fragments have three distinct effects on TCL morphogenesis. Pectic fragments at a concentration of 10 μg/ml inhibit root formation when added to a root-inducing medium containing 15 μM IBA and 0.5 μM kinetin. When pectic fragments (10 μg/ml) are added to a root-inducing medium containing 7 μM IBA and 0.15 μM kinetin, the position of roots changes from a random distribution over the TCL surface to an asymmetric distribution on the basal end of the TCL. Addition of pectic fragments to a medium containing 1.5 μM IBA and 0.9 μM kinetin causes a marked tissue enlargement at the basal end of the TCL and induction of flowers. The flower-inducing activity of the pectic fragments is stable to boiling and protease digestion. Tobacco pectic fragments, like sycamore fragments, induce flower formation on tobacco TCLs. We propose that oligosaccharins (oligosaccharides with biological activity) play a role in plant development. (Supported by NIH F32 GM11857-02 (to D.M.) and DOE DE-FGO9-85ER13425).